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Biochemical Genetics of Blood Coagulation*

JOHN B. GRAHAM

School of Medicine, University of North Carolina, Chapel Hill

THE CHAIRMAN of this symposium asked me to discuss the biochemical genetics of blood coagulation. I assented before I discovered, too late to express my regrets, that this was a formidable job. The literature is very large, and my own experience has been "existential" only with regard to hemophilia. By restricting myself to recent publications suspected of having or purporting to have genetic implications, I was able to limit my reading to about 300 papers. I should warn you not to expect a discussion of blood coagulation as if it were *Neurospora* metabolism. There are, however, some points of genetic interest which I shall attempt to summarize. I have tried to be judicial rather than polemical and hope that my analysis will indicate the direction for future investigations.

It might be worthwhile to outline recent developments in blood coagulation research. This sketch necessarily will be more abbreviated than that of Milstone (61), the historian of ideas in this field. Most biologists have been exposed at one time to the diagram in Figure 1 representing the steps in blood clotting. This has been known as the Morawitz theory since its enunciation in 1904 (62). During the period of the 1930's the Morawitz formulation was useful because primary emphasis was on the development of methods for assaying prothrombin and thrombin, on the concentration and purification of these proteins, and on their metabolism. This was the period when was done the classical work on the relationship of prothrombin to Vitamin K and the identification, purification and synthesis of this vitamin. This culminated, you may remember, in a Nobel prize in 1943 for Edward Doisy and Henrik Dam.

As regards the Morawitz hypothesis, a disquieting note was sounded by workers at the University of Iowa in 1939 (64). They noted that the one-stage prothrombin method gave normal results with infant's plasma while the two-stage method showed marked reduction of prothrombin. They inferred, correctly in retrospect, that circulating plasma contains a "convertibility factor" controlling the rate of transformation of the proenzyme, prothrombin, into active thrombin. They believed that in infant's blood a normal level of the "convertibility factor" was concealing a deficit of prothrombin. This suggestion opened Pandora's box. During the succeeding 15 years, at least seven distinct substances were described, in addition to the already known

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1. PROTHROMBIN + THROMBOPLASTIN + Ca IONS ⟶ THROMBIN

2. FIBRINOGEN + THROMBIN ⟶ FIBRIN

FIG. 1. The Morawitz theory of blood coagulation.

antihemophilic factor (AHF), which might be considered to fit into the category of prothrombin "convertibility factors." These discoveries are summarized in Table 1.

The first discovery was made during the war by blood clotters working independently in several countries (26, 66, 74, 105). They discovered a plasma protein which accelerates the conversion of prothrombin to thrombin. This factor is now variously known as "labile factor," "Factor V," "Accelerator globulin" and "accelerin."

In 1949 another "convertibility factor," distinct from Factor V, was discovered in serum (1). This factor acts with tissue thromboplastin to shorten the prothrombin time, and has been designated by various authors as "SPCA" (serum prothrombin conversion accelerator), "proconvertin," "Factor VII" and "stable factor." Several small families deficient in this factor have been described (51, 53, 67).

In 1952, almost simultaneously in the United States (4, 88) and Great Britain (8), was discovered yet another protein required for normal clotting. This substance was called "plasma thromboplastin component" (PTC) in America and "Christmas factor" in England. I shall use the term "Christmas factor" hereafter because the term "PTC" has been been preempted by geneticists in another connection.

Shortly after the reporting of "Christmas factor", a group in New York City (82) reported still another factor said to be required for normal clotting. They named this substance "plasma thromboplastin antecedent" or "PTA", a term dictated by their conception of the blood clotting mechanism. Pedigree study has been attempted in several families thought to lack this substance (32, 82, 84).

In 1954, some of the American discoverers of "Christmas factor" (93) reported the discovery of a possible additional plasma factor, tentatively named the "fourth plasma thromboplastin component". Also in 1954, a group of Swiss workers reported

TABLE 1. HISTORY OF THE DISCOVERY OF THE "CONVERTIBILITY FACTORS"

	"Convertibility Factors"	
Year of Discovery	Name of normal factor	Name of deficiency disease
<1940	Antihemophilic factor	Hemophilia
1947	Factor V, (accelerin, Ac-globulin, labile factor)	Factor V-deficiency, (Parahemophilia)
1949	SPCA, (Factor VII, Proconvertin, stable factor)	SPCA-deficiency
1952	Christmas factor, (PTC)	Christmas disease, (PTC-deficiency)
1953	PTA	PTA-deficiency
1954	Fourth thromboplastin component	Tetartohemophilia
1954	Factor X	Not known
1955	Hageman factor	Hageman disease
>1955	?	?

the discovery of a new clotting factor which they have called "Factor X" (30). Thus far in 1955, I am aware of the reporting of only one new blood-clotting component, the "Hageman factor" (79), but am informed by a "usually reliable source" that the announcement of another discovery is being prepared in Britain.

How good is the evidence that these "convertibility factors" do in fact exist as separate entities? The answer depends on one's criteria. All of them fall into the category of what has been called the "trace proteins", proteins in such low concentration for example, that their absence cannot be detected by chemical or electrophoretic analysis of plasma. Most of them have not been isolated, purified, and characterized in the colloid chemistry sense. However, I think that there is fairly good physiological evidence for the existence of 7 of the 8 factors listed.

If the history of the discovery of these substances is examined, one can see that, with two exceptions (SPCA and Factor X), they have been discovered by the technic which has been so fruitful in the recent work on human hemoglobin and on human blood groups. That is to say, a "new" disease is segregated by studying exceptional cases, in this context exceptional cases of hemophilia. The implicit operational principle is that in each hereditary disease of blood clotting there is a deficiency of one and only one specific factor, an intuitive "one gene-one enzyme" hypothesis. Thus, for example, when plasma from an apparent case of hemophilia corrects the clotting defect in plasma from an established case of hemophilia, the diseases are assumed to be due to deficiencies of entirely different substances. When the exceptional plasma is found to correct the clotting defect in all other types of hemophilia-like deficient plasmas, it is generally agreed to be unique. Also, it is generally agreed that the patient providing the plasma suffers from a previously undifferentiated disease. It is then inferred that plasma of normal individuals contains a previously unrecognized

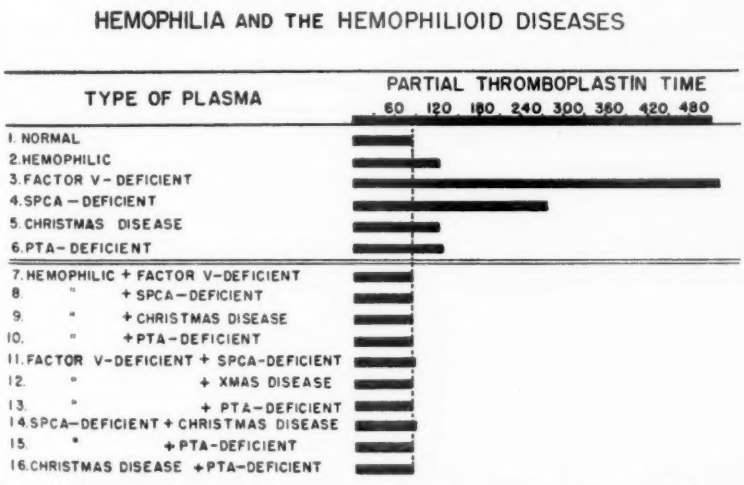

FIG. 2. A physiological experiment showing the corrective effect of each type of deficient plasma on each of the other types of deficient plasma. The broken vertical line projects the clotting time of the normal plasma for comparison.

factor which is missing in the affected person's plasma. This conclusion is conventionally followed by an attempt to isolate the newly discovered factor from normal plasma and characterize it chemically.

An example from our laboratory (Figure 2) will illustrate this general method of analysis. We managed to obtain fresh plasma simultaneously from persons having hemophilia, Factor-V deficiency, SPCA-deficiency, Christmas disease, and PTA-deficiency. All plasmas were tested by a simple clotting test developed in our laboratory which we have named the partial thromboplastin time (48). The partial thromboplastin times of all the abnormal plasmas were prolonged, Lines 2–6. However, the clotting times of the mixtures of equal parts of plasma from each pair of diseases were normal, Lines 7–16. This general type of experiment reproduced by many workers under many different sets of conditions is consistent with the current hypothesis that each disease is caused by a deficiency of a specific clotting factor. This concept has been expressed pictorially in an almost identical fashion by a Swiss investigator recently (46).

Attuned to the times, we generally rewrite the two steps of the Morawitz scheme as enzymatic rather than stoichiometric reactions (Figure 3). It is clear that all of the "convertibility factors" act prior to or during the prothrombin-to-thrombin reaction and are not related to the polymerization of fibrinogen. How each individual substance reacts is less clear. At present it is believed that each of the new factors either participates in the formation of thromboplastin, or acts to accelerate the transformation of prothrombin to thrombin in some other fashion.

You will notice that I have been exceedingly naive thermodyamically in Figure 3, but rather crafty. The first reaction step has been written as though it were catalyzed by an agent which is itself produced as the result of a tenth-order reaction. Even if thromboplastin were clearly established, such a reaction would be highly improbable. It is more likely that these factors react with one another in sets of first, second, and possibly third-order reactions in some definite sequence. The result of

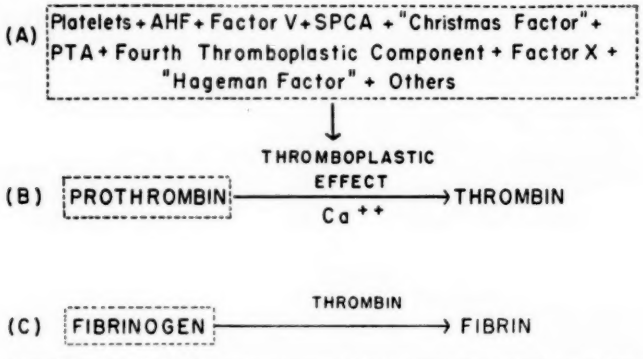

FIG. 3. A modification of the Morawitz theory of blood coagulation which takes into account the discoveries of the past 15 years. This is grossly oversimplified as it does not take into account the undoubted but presently unclear interrelationships of the "convertibility factors".

these interactions I have purposely avoided committing myself on, merely indicating a catalytic action called the "thromboplastic effect." I would opine that during the next decade blood clotters will be as fully occupied in isolating these factors and studying their interrelationships as the nuclear physicists will be with the myriad of subnuclear particles.

There are several facts which I would like to emphasize at this point.

1. The end product of this series of reactions, whatever the sequence, is fibrin. Fibrin is used in the body to stanch bleeding. Whenever fibrin formation is impaired, a hemorrhagic state may result.

2. There are basically four ways now known in which fibrin formation may become impaired:

   a. Reduction in, or physiological inactivity of, any one of the substances contributing to what I have chosen to call the "thromboplastic effect."
   b. Reduction in or physiologic inactivity of prothrombin.
   c. Absence of fibrinogen.
   d. Excess of an inhibitory substance which neutralizes the action of one of the known procoagulants. (I shall not discuss inhibitors further except to say that several types are well known.)

With these facts in mind, I think present knowledge of the genetic control of blood clotting factors may be summarized succinctly.

1. The areas on Figure 3 included within the broken rectangles and designated (A) and (C) are clearly under genetic control; there is good evidence for an hereditary deficiency of the AHF, Factor V, SPCA, Christmas factor, and fibrinogen and fair evidence for an hereditary defect of platelets, PTA, "fourth thromboplastin component" and "Hageman factor."

2. The genetic control of prothrombin synthesis is less clear. There is at present very little evidence supporting an hereditary deficiency of prothrombin, the large literature to the contrary notwithstanding. With the possible exception of Quick's cases (76), the cases of so-called prothrombin deficiency (to my knowledge) have either proved to be SPCA-deficiency, or this possibility has not been excluded. Recently, Seegers and his associates have reported evidence interpreted as showing that SPCA is an altered form of prothrombin (86, 87). If this is confirmed, it may imply that what we now know as "SPCA-deficiency" is, in reality, the hereditary defect of prothrombin which has been so elusive.

In the remainder of this paper, I would like to discuss in more detail four of the blood clotting factors, the four about which genetic knowledge is most sound.

FIBRINOGEN, FIBRIN AND AFIBRINOGENEMIA

Fibrinogen is present in plasma at a higher concentration than any of the other coagulation proteins, 300–500 mg%. The work on fibrinogen is sufficiently advanced that one or another of several relatively pure preparations may be purchased at the pharmacy. Physical chemical studies of the fibrinogen molecule are consistent with a thin rod model about 600 x 50 Å, of molecular weight about 330,000 (89). Under the action of the active enzyme, thrombin, fibrinogen is polymerized to the larger mole-

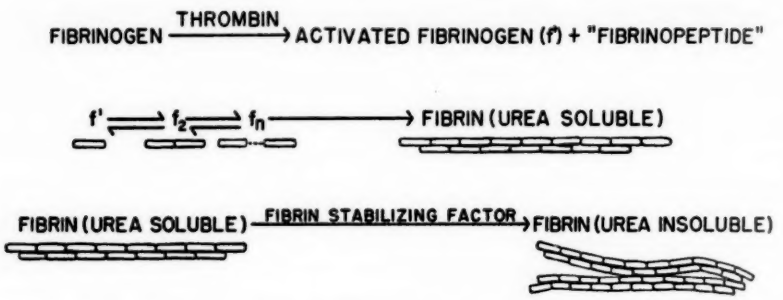

FIG. 4. The formation of fibrin from fibrinogen.

cule, fibrin, molecular weight approximately 5,000,000 or about 16 fibrinogen residues. Studies on the molecular properties of fibrinogen have been reviewed recently by Lorand (55) and by Ferry (28), and are shown schematically in Figure 4.

Thrombin appears to act on fibrinogen by decreasing the net negative charge and redistributing the remainder so that the molecules no longer repel each other. This is accomplished by splitting out from each fibrinogen molecule a negatively charged peptide of molecular weight 4000–8000 which has been named "fibrinopeptide" (55). The less negatively charged "activated fibrinogen" then forms intermediate polymers, of all sizes, reversibly, in many steps. This process terminates in the large fibrin molecule. There is evidence that the fibrin end-product is composed of fibrinogen units linked not only end-to-end but also side-to-side with overlapping ends (28).

Fibrin prepared from purified fibrinogen by the action of purified thrombin is soluble in urea. However, if fibrin forms in plasma, or if plasma (or serum) and calcium ions are added to the mixture of purified thrombin and fibrinogen, the clots become insoluble in urea. This change in solubility led to the obvious hypothesis that plasma contains a "fibrin-stabilizing factor" (55). This factor is believed to promote the formation of side linkages between fibrin molecules. In preliminary studies, the "fibrin-stabilizing factor" has been shown to be a thermolabile protein, constituting only a small fraction of total plasma protein (55).

It has been shown that the "fibrinopeptides" split out of human and bovine fibrinogen by thrombin differ in amino acid composition (55). Also, the terminal amino groups of fibrinogen molecules are different in the two species (54). However, human and bovine fibrinogen molecules are of approximately the same size and shape (89), and the fibrinogens of both species form fibrin with approximately equal speed when acted on by the thrombins of both species. The great similarities and minor differences would imply to geneticists that fertilized human and bovine ova are carrying approximately the same information regarding fibrinogen synthesis. The quite slight species differences in chemical structure appear to have no physiological significance, suggesting that fibrinogen samples from humans *without* bleeding tendencies might show similar chemical differences, perhaps genetically determined. Geneticists might anticipate also that close scrutiny would reveal mutations affecting "fibrinopeptide" and "fibrin-stabilizing factor." Studies on both substances in

human populations are possible in the near future since assay procedures for both are available (55, 56).

Congenital fibrinogen deficiency was described originally in 1920 (77). Theoretically, it is possible that the defect in this condition is not the deficient production of normal fibrinogen, but either excessive destruction or a major alteration in the fibrinogen molecule changing its physical, chemical and physiologic properties. There is evidence which allows rejection of the excessive destruction hypothesis. A fibrinogen half-life of 5.6 days has been found by feeding S^{35} labeled d-l methionine and yeast to normal humans (59), and a half-life of 3.5 days by transfusing labeled fibrinogen (102). It has been shown also that the half-life of normal fibrinogen transfused into afibrinogenemic humans is about 4 days (34, 52). Such close correspondence would not be found if the disease state resulted from excessive destruction.

The possibility of an altered molecule cannot be excluded with the same confidence, since the methods for identifying and measuring the quantity of fibrinogen in plasma depend on its molecular characteristics. The quantity of fibrinogen, 300–500 mg%. is sufficiently small that an abnormal molecular species might be overlooked in the 7,000 mg% pool of plasma proteins. However, since the cases described appear to lack fibrinogen as tested by the three technics of thrombin action, electrophoretic analysis and immunochemical precipitation, it is reasonable to assume that the affected persons simply synthesize little or no fibrinogen.

There are 29 cases of congenital afibrinogenemia in the literature[1], exclusive of Risak's cases (80) which are dubious. Those reported prior to 1954 were well summarized by Frick and McQuarrie (33), and the cases reported since are included in the bibliography (3, 52, 73, 100). Of the patients described, 21 were males and 8 females. In 9 of the 25 matings, the parents were first cousins. The high degree of consanguinity suggesting a recessive gene, it is instructive to look at the data pertaining to carriers. Fibrinogen determinations are available on 17 parents. In only 4 parents was the fibrinogen below 200 mg% and in only one below 100 mg%. Clearly, most heterozygotes are normal overlaps. Furthermore, none of the authors report the variation in normal populations or the internal variation of the fibrinogen methods used. Even more fundamental, it is not clear in any of the studies that precautions were taken to bypass the action of the fibrinolytic enzyme which can cause erroneously low fibrinogen values. It is my opinion that no one has yet clearly shown that the carrier state in this condition can be detected with confidence.

HEMOPHILIA AND THE ANTIHEMOPHILIC FACTOR (AHF)

Classic hemophilia was probably the first hemorrhagic disease recognized, and it is likely that all hemorrhagic diseases at one time were considered cases of hemophilia. There is little doubt, for example, that in Andreasson's inclusive survey in Denmark (5) examples of the other "hemophilioid diseases" (deficiencies of the other "convertibility factors") were included amongst his hemophiliacs. With the diagnostic methods he used, this was inevitable.

Some experimental work was done on hemophilia during the 1930's. It was shown

[1] The actual number of cases is probably less. Several have been reported more than once.

that normal plasma contains a substance, isoelectrically precipitated at pH 5.3, which shortens the clotting time of hemophilic blood both *in vitro* and *in vivo* (68). However, this so-called "antihemophilic globulin" was never satisfactorily purified, and work on it ceased about 1940 to be taken up again only quite recently (7, 104). A significant break-through in the pathogenesis of hemophilia was made in 1939 with the demonstration by Brinkhous (13) that the slow clotting in this disease was due to slow transformation of prothrombin to thrombin. This worker also demonstrated later the essentiality of platelets for coagulation, and that a plasma factor absent in hemophilia reacts with platelets before blood clotting begins (14).

Competing with the concept that hemophilia is a deficiency disease has been the hypothesis that the bleeding tendency results from excess of an inhibitor, anticephalin. A summary of the supporting data for this view was published several years ago (99). Only recently has it been shown that the anticephalin hypothesis is probably erroneous (39, 40), removing this objection to the deficiency hypothesis.

Hemophilia in dogs (29)—severe, sex-linked, and identical with the human disease—has been studied in our laboratory since 1947 (35). The first female indubitably homozygous for hemophilia was a dog (15), and human homozygotes have been seen rarely (43, 60a). Since the breeding of female hemophilic dogs became routine, it has been possible to mate them with hemophilic males, producing as many as 8–10 hemophiliacs in a litter. Two moderately accurate methods for determining the AHF content of various fluids were developed using the hemophilic dogs (37, 48). With these technics, the titre of AHF in normal animals has been shown to have wide species variations (16, 94). The use of dogs for experiments on the AHF has resulted in the discovery of many facts about this factor which have not been ascertainable about the other "convertibility factors". It was discovered in normal dog blood that the antihemophilic factor disappears during coagulation (36). There is also evidence suggesting that the antihemophilic factor participates in a reaction with "Christmas factor" and platelets producing thromboplastic activity (9). This reaction is the basis for the thromboplastin generation test (10), a new blood clotting method well adapted to large-scale clinical studies. A reaction between AHF and platelets has been suggested also by the simultaneous decrease in both elements after cold injury to soft tissues or injection of thromboplastin (18).

It has been shown that severe liver damage and dicoumarolization do not affect the AHF level (37, 71). It has been shown also that AHF remains at a normal level during life in dogs given a lethal dose of X-irradiation (70). These data seem to eliminate the liver and lymphoid tissue as sites of formation of this substance. Splenectomy has been shown both in dogs and humans not to affect the clinical course of hemophilia, nor alter the level of AHF in normal dogs (41). Pancreatectomy also seems not to affect AHF (71).

Recently it has been found that d-l ethionine depresses AHF levels in rats. Severe deterioration of the bone marrow cells was noted in these animals also. It was provisionally concluded that the elusive site of AHF synthesis had been located in the reticulo-endothelial cells (71). While this is a logical inference from the evidence, it may not be the correct one. It seems to me that there is an alternate explanation of this important observation which is equally likely. It is probable that d-l ethionine

depresses AHF by competitively inhibiting incorporation of methionine into the AHF molecule and blocking AHF production. It is possible that reticulo-endothelial deterioration and AHF depression are merely coincidental. Protein synthesis is high in the marrow, and this may make these cells particularly susceptible to destruction when adequate methionine is not available. It is interesting that in these experiments the liver cells also showed severe deterioration, because the liver is also the site of large scale protein synthesis. It is possible that the difficulty in pin-pointing the site of synthesis of AHF arises because AHF is produced in many parts of the body. This possibility would be consistent with the fact that production has been interfered with only once and then by an agent which affects all cells.

The turnover rate of the AHF has been determined recently by transfusing both normal and hemophilic dogs with plasma and plasma fractions (49). An extraordinarily short half-life, 2 hours, has been found in both canine genotypes. The agreement of the turnover data in the two dog types is consistent with the conventional impression that the hemophilia gene acts by decreasing the production of AHF rather than by producing an inhibitor which neutralizes AHF. More recently, preliminary data have suggested a half-life of about 4 hours in hemophilic humans (19), a matter of great therapeutic importance.

Normal plasma is being fractionated for AHF in many laboratories. In our laboratory canine AHF has been purified 100 times (104). In England a similarly potent bovine AHF has been obtained (7) and is being used for intravenous therapy in humans (57). In this connection it is to be hoped that bovine AHF proves to be no more antigenic to humans than bovine thrombin (98).

The clinical severity of hemophilia varies widely. Even in inbred hemophilic dogs, all transmitting the same hemophilia gene, there is considerable variation (35). Very mild human hemophilia, diagnosed only by a quantitative AHF assay, has been described in detail in one pedigree (38). This pedigree and the observation that the antihemophilic activity of plasma from normal persons varies widely has led to the postulation of an allelomorphic series of genes at the hemophilia locus (38). The difficulties with this hypothesis are exemplified by the observations shown in Table

TABLE 2. COMPARISON OF ANTIHEMOPHILIC FACTOR LEVELS OF HETEROZYGOTES AND HEMIZYGOTES FOR THE TWO TYPES OF HEMOPHILIA

Type of Hemophilia	Level of Antihemophilic Factor (% of control)					
	Heterozygous females			Hemizygous males		
	No.	Mean	Sum of squares	No.	Mean	Std. dev.
Mild hemophilia						
Type "A"	4	70%	588	6	19%	5%
Type "B"	6	42%	294			
Classic hemophilia	4	116%	15,553	—	<5%	—

"t" test of the differences in group means of the three types of heterozygotes.

1. Mild hemophilia Type "A" v. Mild hemophilia Type "B": $t = 4.285$, 8 d.f., $.01 > P$.
2. Mild hemophilia Type "A" v. Classic hemophilia: $t = 1.254$, 6 d.f., $.3 > P > .2$.
3. Mild hemophilia Type "B" v. Classic hemophilia: $t = 2.576$, 8 d.f., $.05 > P > .02$.

2. It has not been possible to explain by the multiple allele hypothesis why approximately half the women carrying one gene for mild hemophilia and a normal allele have significantly lower plasma AHF levels than women carrying a normal gene and one gene for severe hemophilia. Incidentally, there are no overlaps between the Type "B" and classic heterozygotes. Another group of workers facing a similar problem recently in another connection, have theorized that more than one locus is concerned with AHF production (78). This is certainly reasonable when one considers the probable number of steps in the synthesis of a protein molecule. However, more information will be required about the action of the hemophilia gene before this paradox will be understood.

It should be emphasized that it has been only half the carriers in one pedigree of mild hemophilia who have been categorized with certainty by the AHF assay. The mistaken inclusion of carriers for the "hemophilioid diseases", some of which are dominantly inherited, with hemophilia carriers has confused the problem of carrier detection in earlier studies (5, 91). In several recent attempts at detection of carriers for classic hemophilia (10, 38, 78) normal overlaps have uniformly prevented carrier identification.

FACTOR V (LABILE FACTOR, AC-GLOBULIN) AND PARAHEMOPHILIA

Factor V, the first "convertibility factor" described, other than the AHF, was discovered independently in four widely separated laboratories during the communications breakdown of World War II. The four discoveries were made, interestingly, in three quite different contexts. In America, Quick deduced, after several preliminary attempts (74, 75), that the restorative effect of fresh, prothrombin-free plasma on aged, apparently prothrombin-deficient plasma, was due to an unidentified plasma factor. The easy deterioration of this unidentified factor in the refrigerator suggested to him the name "labile factor."

Owren, elucidating the clotting defect of a female "hemophiliac" in Norway (66), discovered that a plasma factor, distinct from the AHF, would correct his patient's clotting anomaly. He named this substance Factor V (Factors I–IV being his shorthand designation for thromboplastin, calcium ions, prothrombin and fibrinogen). He named the deficiency disease "parahemophilia" because of its clinical similarity to hemophilia.

Ware, Guest and Seegers in the United States (105) discovered that prothrombin was progressively more difficult to convert to thrombin as it was purified. They discovered that prothrombin-free plasma and serum contained a substance which promoted the conversion of purified prothrombin to thrombin. They showed further that the degree of acceleration was proportional to the amount of the new factor added to the system, but that the final yield of thrombin (within limits) was independent of the concentration of this newly discovered substance. They named the factor "accelerator-globulin", because they believed it to be a catalyst rather than a direct participant in the prothrombin-to-thrombin reaction.

The Australians, Fantl and Nance, also discovered the same accelerator by studying the activation of purified prothrombin (26), coming to essentially the same conclusions as Ware, Guest and Seegers.

Factor V has been shown repeatedly to deteriorate on storage. The rate of this deterioration is more rapid as the temperature increases, is more rapid in oxalated than citrated plasma, is more rapid as platelets increase in number, and is more rapid in ordinary glass containers than siliconized ones when platelets are present (24). Preliminary purification suggests that Factor V is a thermolabile globulin (66) which decreases during clotting (95). The coumarin drugs have little effect on Factor V production (25, 63). The turnover rate has not been ascertained, but the half-life is certainly less than 3 days (65). Chloroform intoxication causes a reduction in plasma Factor V (97), but splenectomy does not (41). These experiments suggest that this globulin is fabricated chiefly in the liver, and that little if any is produced in the spleen. When Factor V is congenitally deficient, administration of neither Vitamin K (2), protamine sulfate (12) nor aminophylline (12) will reverse this state, although aminophylline causes an increase of Factor V in normal dogs (60).

The corresponding deficiency disease, parahemophilia, resembles hemophilia clinically. Of the 18 cases reported (2, 11, 12, 23, 31, 44, 65, 67, 85, 96, 103) not more than 4 were sporadic, the remainder being familial. Ten patients were males, eight females. Recent study of a large consanguineous Afrikaner kindred using a semi-quantitative assay procedure suggests that those clinically affected are homozygous for the mutant gene (44). Using this assay technic, all parents and all children of clinically affected persons were found to have a significant reduction of Factor V. In the critical sibship, the distribution of siblings was—3 affected: 4 partially affected: 2 normal—consistent with a recessive gene. It is interesting to note that the action of the single parahemophilia gene, a clinical recessive, is detectable. This emphasizes the thesis that even a recessive gene has its effect, and implies in this instance that population surveys for Factor V deficiency are possible if one uses the proper assay procedure. Ordinary one-stage prothrombin tests are clearly not suitable for this purpose. Clinical cases of parahemophilia are certainly rarer than cases of hemophilia or Christmas disease. However, if affected individuals are actually homozygous, the Hardy-Weinberg Law would lead geneticists to wonder whether the gene frequency may not prove to be equal to or greater than that of hemophilia and Christmas disease.

CHRISTMAS FACTOR AND CHRISTMAS DISEASE

This disease was probably discovered and reported with the wrong interpretation by Pavlovsky in 1947 (69). He noted that the plasmas of two hemophiliacs, indistinguishable by his tests, were mutually corrective. The next reports were almost simultaneous in 1952; one by a group in New York City (88), and the other by a group in San Francisco (4). The Californians named the disease "PTC-deficiency", the term in common use today in the United States. Our British collegues published their studies several months later, during Christmas week 1952 (8). The highly improbable coincidence of having a patient with an unusual disease and the surname Christmas and publishing during Christmas week tickled the public fancy and the term "PTC" has never made headway outside the United States.

Eighteen papers reporting 69 undoubted cases of "Christmas disease" have been published since 1951, (4, 6, 8, 21, 22, 27, 32, 42, 50, 58, 72, 78, 81, 83, 88, 90, 92,

TABLE 3. FREQUENCY OF HEMOPHILIA AND CHRISTMAS DISEASE

Total cases surveyed and reclassified	= 247
Hemophilia	= 207
Christmas disease	= 40
Frequency of hemophilia in Danish males (Andreasson's survey)	= 4.45×10^{-4}
Adjusted frequencies for Danish males	
Hemophilia	= 3.73×10^{-4}
Christmas disease	= 7.16×10^{-5}

101). All cases show essentially the same features. A hemorrhagic disease resembling mild to moderately severe hemophilia clinically, is shown not to be hemophilia by study of the clotting anomaly. Where good pedigrees are available, a sex-linked mode of transmission is noted. The condition is not as completely recessive as hemophilia. For example, four of the seven mothers who have been tested adequately, have shown a clear diminution of Christmas factor (17, 78, 83). The disease is easily diagnosed with the partial thromboplastin time (48), the thromboplastin generation test (10), or the classical method, recalcification of mixtures of the unknown plasma with plasma samples from the other hemophilioid diseases. Recently it has been shown that the coumarin drugs depress "Christmas factor" production (90).

A fairly good estimate of the frequency can be given (Table 3). In nine different laboratories, from Switzerland to Australia (8, 27, 32, 47, 58, 81, 83, 92, 101) workers have surveyed the available "hemophiliacs", reclassifying 247 of them as classic hemophilia or Christmas disease. Of these, 207 were deficient in AHF, 40 in Christmas factor. Thus, 16.1% or approximately 1:6 of all "hemophiliacs" were in fact suffering from Christmas disease. This implies that Andreasson's estimate of the frequency of hemophilia in Danish males probably should be refined by reducing it from 4.45×10^{-4} to 3.73×10^{-4} before the next attempt at calculation of the mutation rate. It also allows the estimation of the frequency of Christmas disease in Danish males as about 7.16×10^{-5}.

A final bit of irony. It has been shown recently (45) that one of the large hemophilia pedigrees in the collection of Bulloch and Fildes (20), the Tenna kindred, is in reality Christmas disease. This might have been anticipated, as Christmas disease is much less severe than classic hemophilia on the average. One wonders whether the same thing is not true also of the Mampel (20) and some of the other large and famous hemophilia kindreds.

SUMMARY AND CONCLUSIONS

An attempt has been made to analyze recent developments in blood coagulation using genetic categories. From the standpoint of biochemical genetics, reliable data are scant, but a small beginning has been made with fibrinogen and the antihemophilic factor. The lack of progress along genetic lines in other areas may be attributed to:

1. the rarity of the newly discovered hemophilioid diseases,

2. concentration by most workers on identifying and subclassifying these diseases,

3. intense interest in the biochemical properties of the newly described factors and their roles in clotting, and

4. apparent failure to recognize the importance of genetic studies. This last opinion is implied by the failure to develop assay procedures sufficiently reliable for family studies, and improper application of such methods as are available. Specifically, one is impressed by the exhaustive studies on probands and almost complete neglect of relatives.

In my opinion, significant genetic advances will be made rather quickly when the workers in the field appreciate the possibilities.

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The Detection and Estimation of Linkage Between the Genes for Elliptocytosis and the Rh Blood Type[1]

NEWTON E. MORTON

University of Wisconsin

RECENTLY SEVERAL INVESTIGATORS have reported that the genes for elliptocytosis and the Rh system may be closely linked (Goodall et al., 1953, 1954; Lawler and Sandler, 1954; Marshall et al., 1954). Other studies, however, did not suggest linkage (Chalmers and Lawler, 1953; Holländer, 1954; Fujii et al., 1955; Vandepitte and Louis, 1955). The data on this subject are unusually extensive, and they provide strong evidence of an autosomal linkage in man. Since these pedigrees also exemplify some of the difficulties encountered in the study of human linkage, it may be profitable to analyse them more closely.

THE MATHEMATICAL METHOD

The analysis is based on the probability ratio test, which in sequential samples is more efficient and gives a more exact significance level than alternative criteria for the detection of linkage (Morton, 1955). It will now be shown that this test may easily be applied to pedigree data, and that it leads to simple heterogeneity tests and estimation procedures.

Let θ be the probability of recombination between two given gene loci and $f(y_i\,;\theta)$ be the probability of occurrence of the i^{th} family or pedigree, the successive samples $y_1, y_2, \cdots$, etc. being independent. The test statistic is

$$z_i = \log_{10} \frac{f(y_i;\, \theta_1)}{f(y_i\,;\tfrac{1}{2})}, \qquad (\theta_1 < \tfrac{1}{2})$$

where the null hypothesis to be tested is that $\theta = \frac{1}{2}$ and the formal alternative is $\theta = \theta_1$. The test employs two positive numbers, $A > 1$ and $B < 1$, and continues until $\sum z_i \geq \log A$, in which case the null hypothesis is rejected, or $\sum z_i \leq \log B$, whereupon the alternative hypothesis is rejected. Wald and Wolfowitz (1948) have shown that this procedure requires on the average fewer observations than any other test having the same Type I and Type II errors. Earlier Wald (1947) had shown that these errors are approximately

$$\alpha \simeq \frac{1 - B}{A - B}, \qquad \beta \simeq \frac{B(A - 1)}{A - B},$$

that the approximations are accurate when the excess of $\sum z_i$ over the boundary $\log A$ or $|\log B|$ at the termination of the test is negligible, and that the inverse

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formulae

$$A \simeq \frac{1-\beta}{\alpha} \qquad B \simeq \frac{\beta}{1-\alpha}$$

cannot lead to any appreciable increase of α or β when both are small.

Unless there is other evidence that two genes are linked, the low prior probability of linkage imposes a stringent significance level ($\alpha = .001$). The choice of θ_1 is determined by the amount of data which can be collected by investigators with a "reasonable" effort. With $\log A = 3$, $\log B = -2$ ($\alpha = .001$, $\beta = .01$), the choices $\theta_1 = .20$ and $\theta_1 = .30$ correspond to expected sample numbers of about 70 and 360 double backcross sib pairs, respectively, and also to about 70 and 360 units of information in Finney's system of u scores (Finney, 1940; Morton, 1955).

At the termination of the test, especially if the null hypothesis has been rejected, we may with profit perform a homogeneity test or make other checks against heterogeneous data and causes other than linkage. In what follows it is assumed that no serious error will be introduced by using large-sample homogeneity tests and estimation procedures (Anscombe, 1952). In the long run this is presumably justified, since detection of linkage leads to collection of more data for estimating linkage. On this assumption, a convenient homogeneity criterion is the logarithm of the likelihood ratio,

$$(1) \qquad \mathfrak{L} = 2(\log_e 10)\left(\sum_{i=1}^{n} \hat{z}_i - \hat{Z}\right) = 4.605(\Sigma\hat{z}_i - \hat{Z})$$

where $\hat{z}_i$ denotes the maximum of z_i in the interval $0 \leq \theta \leq \frac{1}{2}$, $\hat{Z}$ is the maximum of $\sum z_i$ in the same interval, n is the number of samples (families or groups of families), and logarithms to the base 10 are used for the z_i. In the limit for large samples, $\mathfrak{L}$ has the χ^2 distribution with $n - 1$ degrees of freedom, if the data are homogeneous (Wald, 1943). Asymptotically, $\mathfrak{L}$ is equivalent to the maximum likelihood homogeneity test, but with small samples it may be more exact (Fisher, 1950; Cochran, 1952). For either test, small families will usually be grouped to avoid decisions based on extremely small subclass numbers. In the limit for large bodies of data, $4.605\,\hat{Z}$ has the χ^2 distribution with one degree of freedom, if $\theta = \frac{1}{2}$.

If there is evidence for linkage and the data seem fairly homogeneous, an estimate of the recombination fraction θ is in order; the maximum likelihood estimate $\hat{\theta}$ is good on the basis of its asymptotic properties. Given the z_i for a few values of θ, $\hat{\theta}$ and the $\hat{z}_i$ can be determined by interpolation. Usually graphical interpolation will be sufficiently accurate.

The method of deriving a confidence interval for θ depends on the amount of data. If the material is extensive enough so that $\Pi f(y_i\,;\,\theta)$ is nearly normal, then $Z = \sum z_i$ is approximately of the parabolic form

$$Z = a + b\theta + c\theta^2,$$

where

$$b = M\hat{\theta}/\sigma^2, \qquad c = -M/2\sigma^2, \qquad M = \log_{10}e = .4343.$$

Any three points, say (θ_1, Z_1), (θ_2, Z_2), and (θ_3, Z_3), are sufficient to determine a, b, and c. Let these points be given. Then the large-sample confidence interval for θ is

$$\hat{\theta} - t\sigma < \theta < \hat{\theta} + t\sigma,$$

where t is a standard normal deviate with confidence coefficient $1 - \alpha$ and

$$(2) \qquad \sigma^2 = MQ/2S, \quad \hat{\theta} = \theta_3 + R/2S, \quad \hat{Z} = Z_3 + R^2/4SQ,$$

$$Q = \begin{vmatrix} \dot{\theta}_1 & \dot{\theta}_2 \\ \dot{\theta}_1^2 & \dot{\theta}_2^2 \end{vmatrix}, \quad R = \begin{vmatrix} \dot{Z}_1 & \dot{Z}_2 \\ \dot{\theta}_1^2 & \dot{\theta}_2^2 \end{vmatrix}, \quad S = \begin{vmatrix} \dot{Z}_1 & \dot{Z}_2 \\ \dot{\theta}_1 & \dot{\theta}_2 \end{vmatrix},$$

and $\dot{\theta}_1 = \theta_1 - \theta_3$, $\dot{Z}_1 = Z_1 - Z_3$.

In small samples a suitable transformation of θ will make the distribution of Z more nearly parabolic. This procedure is approximate in small samples, but a precise confidence interval is usually not required. Other small-sample interval estimates are available if the data are homogeneous (Haldane and Smith, 1947).

THE PROBABILITIES OF THE OBSERVED PEDIGREES

Hereditary elliptocytosis (ovalocytosis) is a highly penetrant, "dominant" trait. Since the condition is rare, matings between heterozygotes are exceedingly rare, and since affected individuals are not grossly unfit, nearly all of them are derived, not from a mutant gamete, but by segregation from a heterozygous parent. Therefore, practically all matings of interest are backcrosses, El/el × el/el, where El and el are respectively the gene for elliptocytosis and its normal allele. In extensive pedigree material, there are few exceptions to the rule that an affected individual has one, and only one, affected parent (Neel, 1953), and there are at most two exceptions in the linkage material. In pedigree B (fig. 9), the individual II.4 does not manifest elliptocytosis, although she has affected offspring and sibs and must be assumed to be El/el. In pedigree Ae (fig. 10), IV.2 is the affected child of untested first cousins, and may be El/El. Treating him as El/el does not appreciably affect the evidence on linkage in this pedigree, since he has no tested full sibs and, being cde/cde, is not an informative parent. With these provisions, we may accept the phenotypes of the individuals and their relations as given, neglecting mutation, illegitimacy, and errors of technique, recording, or penetrance, and assuming that coupling and repulsion are equiprobable in parents of unknown linkage phase. Failure of any of these assumptions can only dilute the evidence for linkage.

For a preliminary test in pedigree material, attention will usually be confined to families of known parental genotype, distinguishing between matings of known and unknown parental phase. These "certain" families may easily be analysed from published tables of z scores (Morton, 1955). In the present case, however, because of the clear significance of linkage in several of the pedigrees, it seems best to extract all the information from these data by computing the complete pedigree probabilities, using the population Rh gene frequencies and the postulate of random

mating when a genotype is unknown. Mourant (1954) gives the required frequencies. Pedigrees 1–7 are English, B and Ae are Swiss, S. S. and M. K. are Japanese, and J. P. N. and J. M. L. are African Negro. Pedigree R is American of unspecified ancestry, but the genotypes are known.

Pedigree 3 (fig. 3) will illustrate the computations. The only Rh gene complexes which appear to be segregating are R_1, R_2, and r (with frequencies .4076, .1411, and .3886, respectively), which together account for 94% of the English Rh gene frequencies. Certain of the rare Rh complexes are indistinguishable from R_1 and R_2 in some of the individuals, but the only effect of this is to modify very slightly the probabilities for the children of III.13. Since this will not appreciably affect the evidence on linkage, we may assume that only the three likely complexes are involved in this pedigree.

With this simplification, the mating II.5 × II.6 must, from its progeny, be $R_1r \times R_2r$. Since it is not known which parent carries the elliptocytosis gene, there are four equiprobable conditions to be considered:

1. $R_1El/r \times R_2r$
2. $R_1/rEl \times R_2r$
3. $R_1r \times R_2El/r$
4. $R_1r \times R_2/rEl$

On the first hypothesis, III.18, III.20, and V.11 must be nonrecombinants, and III.10, III.16, III.22, IV.22, and IV.23, recombinants. The conditional probability so far is $(\frac{1}{2})^3(\frac{1}{4})^5\theta^5(1-\theta)^3$, the factor $\frac{1}{2}$ or $\frac{1}{4}$ arising when the unaffected parent is homozygous or heterozygous, respectively, at the Rh locus. The individual III.12 may be R_1El/R_2, R_1El/r, or rEl/R_2, since we know that she is Rh positive and that she cannot be R_2El/r or R_2El/R_1 if her affected parent was R_1r. The probabilities of the three possible conditions are $\frac{1}{4}(1-\theta)$, $\frac{1}{4}(1-\theta)$, and $\frac{1}{4}\theta$, respectively. In the first case, III.13 may be either R_1r or R_2r; in the second, he may be either R_1R_2 or R_2r; in the third, he may be either R_1R_1 or a heterozygote between R_1 and some other complex not transmitted to his progeny. The corresponding probabilities are:

III.12	III.13	Probability
R_1El/R_2	R_1r	$2(.4076)(.3886)(\frac{1}{2})(\frac{1}{4})^{10}\theta^6(1-\theta)^5$
R_1El/R_2	R_2r	$2(.1411)(.3886)(\frac{1}{2})(\frac{1}{4})^{10}\theta^8(1-\theta)^3$
R_1El/r	R_1R_2	$2(.4076)(.1411)(\frac{1}{2})(\frac{1}{4})^{10}\theta^4(1-\theta)^7$
R_1El/r	R_2r	$2(.1411)(.3886)(\frac{1}{2})(\frac{1}{4})^{10}\theta^8(1-\theta)^3$
rEl/R_2	R_1R_1	$(.4076)^2(\frac{1}{2})^7(\frac{1}{4})^4\theta^7(1-\theta)^4$
rEl/R_2	R_1/non-R_1	$2(.4076)(.5924)(\frac{1}{2})(\frac{1}{4})^{10}\theta^7(1-\theta)^4$

The probability of the whole kindred, on this hypothesis as to the genotypes of II.5 and II.6, is therefore

$$f_1 = (\tfrac{1}{4})^{17}[.3168\theta^{11}(1-\theta)^8 + .1097\theta^{13}(1-\theta)^6 + .1150\theta^9(1-\theta)^{10} + .1097\theta^{13}(1-\theta)^6 + 11.1157\theta^{12}(1-\theta)^7].$$

Similar calculations for the other three possible genotypes of II.5 and II.6 give

$$f_2 = (\tfrac{1}{4})^{17}[.3168\theta^{11}(1-\theta)^8 + .2194\theta^{13}(1-\theta)^6 + .1150\theta^9(1-\theta)^{10} + 11.1157\theta^{10}(1-\theta)^9]$$

$$f_3 = (\tfrac{1}{4})^{17}[11.4325(1-\theta)^{19} + .1097\theta^6(1-\theta)^{13} + .1150\theta^{11}(1-\theta)^8 + .1097\theta^7(1-\theta)^{12}]$$

$$f_4 = (\tfrac{1}{4})^{17}[11.4325\theta^6(1-\theta)^{13} + .1097\theta^{12}(1-\theta)^7 + .1150\theta^{15}(1-\theta)^4 + .1097\theta^{11}(1-\theta)^8]$$

The unconditional probability is $\frac{1}{4}(f_1 + f_2 + f_3 + f_4)$. Summing and rounding off the last three decimal places, we find

$$f(y;\theta) \propto 114(1-\theta)^{19} + 115\theta^6(1-\theta)^{13} + \theta^7(1-\theta)^{12} + 2\theta^9(1-\theta)^{10} + 111\theta^{10}(1-\theta)^9 + 9\theta^{11}(1-\theta)^8 + 112\theta^{12}(1-\theta)^7 + 4\theta^{13}(1-\theta)^6 + \theta^{15}(1-\theta)^4.$$

$$z = \log \frac{f(y;\theta)}{f(y;\tfrac{1}{2})} = \log \frac{2^{19}}{469}\{114(1-\theta)^{19} + \cdots + \theta^{15}(1-\theta)^4\}.$$

In the same way, we can calculate the z scores for the other pedigrees.

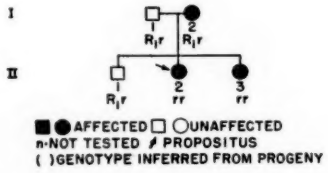

FIG. 1. Pedigree 1 (Chalmers and Lawler, 1953)

PEDIGREE 1 (fig. 1)

$$z = \log 2\{\theta^2 + (1-\theta)^2\}$$

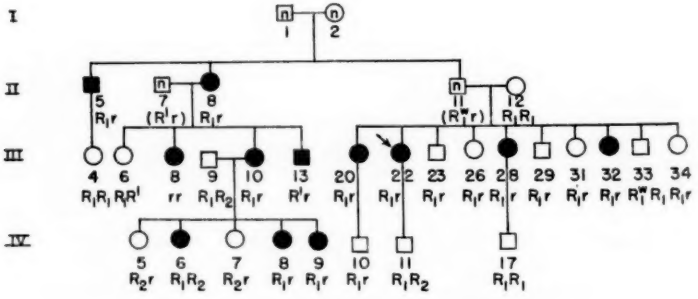

FIG. 2. Pedigree 2 (Chalmers and Lawler, 1953)

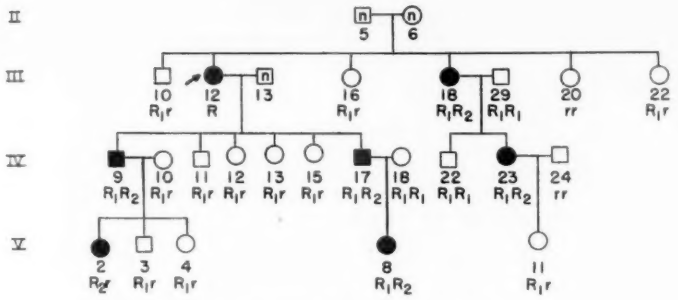

FIG. 3. Pedigree 3 (Goodall et al., 1953)

PEDIGREE 2 (fig. 2)

$$\text{II.11, 12: } z = \log 2^{10}\theta^5(1-\theta)^5$$

$$\text{other: } \quad z = \log \frac{2^{10}}{.9767}(1 - .0466\theta)\theta^2(1-\theta)^6\{\theta^2 + (1-\theta)^2\}$$

$$\text{all data: } z = \log \frac{2^{25}(1 - .0466\theta)}{52(.9767)}\{3\theta^8(1-\theta)^{17} + 2\theta^8(1-\theta)^{14} + 2\theta^9(1-\theta)^{16}$$

$$+ 2\theta^{10}(1-\theta)^{15} + 3\theta^{11}(1-\theta)^{14} + 13\theta^{12}(1-\theta)^{13} + 13\theta^{13}(1-\theta)^{12}\}$$

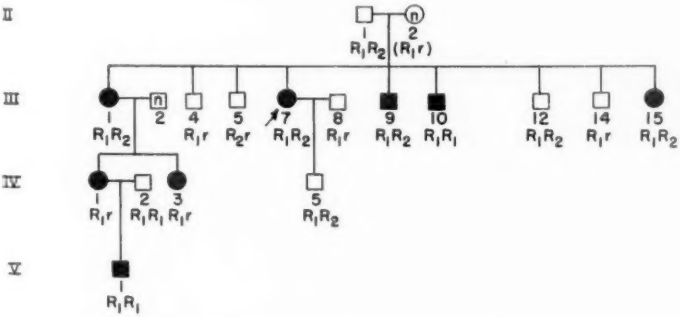

FIG. 4. Pedigree 4 (Goodall et al., 1954)

PEDIGREE 4 (fig. 4)

$$z = \log 2^{12}\{\theta(1-\theta)^{12} + \theta^8(1-\theta)^5\}$$

PEDIGREE 5 (fig. 5)

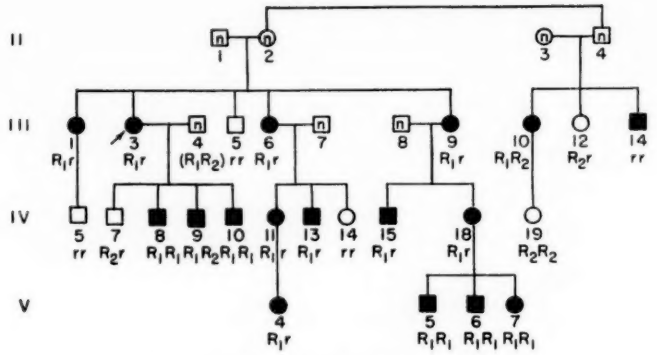

FIG. 5. Pedigree 5 (Lawler and Sandler, 1954)

$$z = \log \frac{2^{20}}{39168}\{810\theta(1-\theta)^{19} + 324\theta(1-\theta)^{18} + 180\theta(1-\theta)^{17} + 72\theta(1-\theta)^{16} + 90\theta^{3}(1-\theta)^{17} + 72\theta^{3}(1-\theta)^{16} + 40\theta^{3}(1-\theta)^{15} + 24\theta^{3}(1-\theta)^{14} + 90\theta^{4}(1-\theta)^{15} + 20\theta^{4}(1-\theta)^{13} + 90\theta^{5}(1-\theta)^{15} + 432\theta^{5}(1-\theta)^{14} + 20\theta^{5}(1-\theta)^{13} + 104\theta^{5}(1-\theta)^{12} + 1800\theta^{6}(1-\theta)^{14} + 558\theta^{6}(1-\theta)^{13} + 440\theta^{6}(1-\theta)^{12} + 176\theta^{6}(1-\theta)^{11} + 90\theta^{7}(1-\theta)^{13} + 324\theta^{7}(1-\theta)^{12} + 120\theta^{7}(1-\theta)^{10} + 360\theta^{8}(1-\theta)^{12} + 378\theta^{8}(1-\theta)^{11} + 80\theta^{8}(1-\theta)^{10} + 76\theta^{8}(1-\theta)^{9} + 4\theta^{8}(1-\theta)^{4} + 180\theta^{9}(1-\theta)^{11} + 522\theta^{9}(1-\theta)^{10} + 80\theta^{9}(1-\theta)^{9} + 100\theta^{9}(1-\theta)^{8} + 10\theta^{9}(1-\theta)^{3} + 180\theta^{10}(1-\theta)^{10} + 846\theta^{10}(1-\theta)^{9} + 40\theta^{10}(1-\theta)^{8} + 216\theta^{10}(1-\theta)^{7} + 18\theta^{10}(1-\theta)^{4} + 4\theta^{10}(1-\theta)^{2} + 1170\theta^{11}(1-\theta)^{9} + 378\theta^{11}(1-\theta)^{8} + 260\theta^{11}(1-\theta)^{7} + 72\theta^{11}(1-\theta)^{6} + 45\theta^{11}(1-\theta)^{3} + 180\theta^{12}(1-\theta)^{8} + 396\theta^{12}(1-\theta)^{7} + 40\theta^{12}(1-\theta)^{5} + 18\theta^{12}(1-\theta)^{2} + 270\theta^{13}(1-\theta)^{7} + 234\theta^{13}(1-\theta)^{6} + 40\theta^{13}(1-\theta)^{5} + 52\theta^{13}(1-\theta)^{4} + 180\theta^{14}(1-\theta)^{6} + 108\theta^{14}(1-\theta)^{5} + 80\theta^{14}(1-\theta)^{4} + 16\theta^{14}(1-\theta)^{3} + 90\theta^{15}(1-\theta)^{5} + 162\theta^{15}(1-\theta)^{4} + 20\theta^{15}(1-\theta)^{3} + 180\theta^{16}(1-\theta)^{4} + 72\theta^{16}(1-\theta)^{3} + 90\theta^{17}(1-\theta)^{3}\}$$

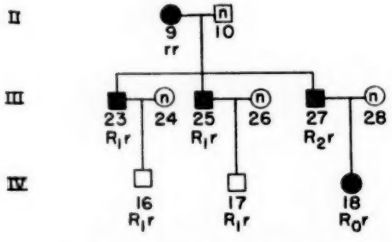

FIG. 6. Pedigree 6 (Lawler and Sandler, 1954)

PEDIGREE 6 (fig. 6)

$$z = \log \frac{2^{3}}{6339}\{1661\,\theta^{2}(1-\theta) + 3168\theta\,(1-\theta)^{2} + 1510\,(1-\theta)^{3}\}$$

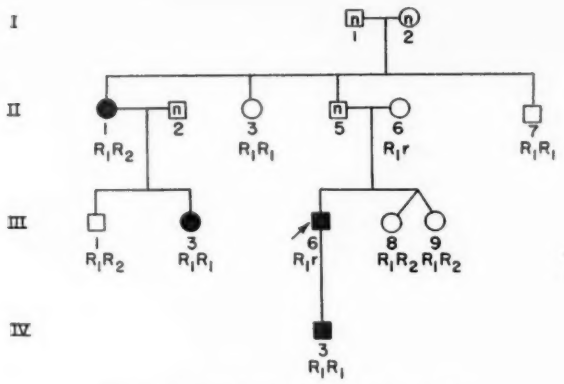

FIG. 7. Pedigree 7 (Lawler and Sandler, 1954)

PEDIGREE 7 (fig. 7)

$$z = \log \frac{2^9}{8097}\{147(1-\theta)^5 + 16(1-\theta)^4 + 521\theta^2(1-\theta)^7 + 58\theta^2(1-\theta)^6$$
$$+ 124\theta^2(1-\theta)^5 + 130\theta^3(1-\theta)^5 + 16\theta^3(1-\theta)^4 + 1314\theta^4(1-\theta)^5$$
$$+ 167\theta^4(1-\theta)^4 + 203\theta^5(1-\theta)^4 + 11\theta^5(1-\theta)^3 + 142\theta^7(1-\theta)$$
$$+ 16\theta^7 + 1275\theta^8(1-\theta) + 140\theta^8\}$$

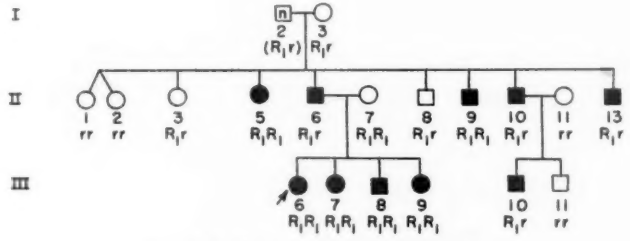

FIG. 8. Pedigree R (Marshall et al., 1954)

PEDIGREE R (fig. 8)

$$z = \log 2^9\{(1-\theta)^{12} + \theta^3(1-\theta)^9 + \theta^5(1-\theta)^7$$
$$+ \theta^6(1-\theta)^5 + \theta^8(1-\theta)^4 + \theta^9(1-\theta)^2\}$$

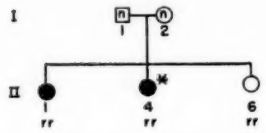

FIG. 9. Pedigree B (Holländer, 1954)

* Elliptocytosis not expressed, but inferred from affected progeny.

PEDIGREE B (fig. 9)

$$z = \log \frac{2^2}{73} \{14 + 17\theta(1 - \theta)\}$$

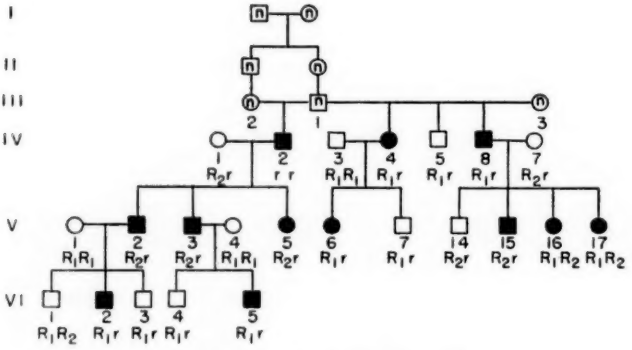

FIG. 10. Pedigree Ae (Holländer, 1954)

PEDIGREE Ae (fig. 10)

$$z = \log \frac{2^{13}}{99} \{6\theta^5(1 - \theta)^8 + 26\theta^6(1 - \theta)^9 + 3\theta^6(1 - \theta)^7 + 17\theta^6(1 - \theta)^5$$
$$+ 16\theta^7(1 - \theta)^8 + 18\theta^8(1 - \theta)^7 + 3\theta^8(1 - \theta)^5 + 16\theta^9(1 - \theta)^6\}$$

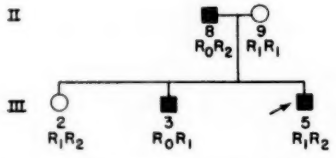

FIG. 11. Pedigree S. S. (Fujii et al., 1955)

PEDIGREE S. S. (fig. 11)

$$z = \log 2^2\theta(1 - \theta)$$

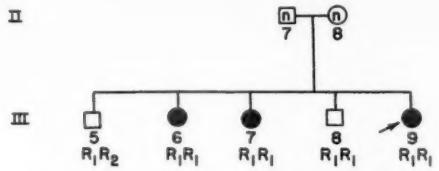

FIG. 12. Pedigree M. K. (Fujii et al., 1955)

PEDIGREE M. K. (fig. 12)

$$z = \log \frac{2^3}{103} \{6 + 97\theta(1-\theta)^4 + 6\theta(1-\theta)^3 + \theta^2(1-\theta)^2 + 6\theta^3(1-\theta) + 97\theta^4(1-\theta)\}$$

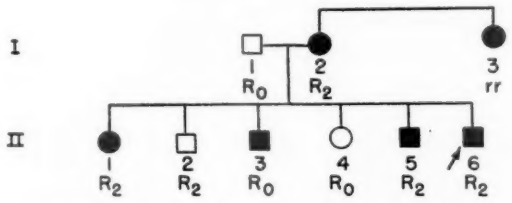

FIG. 13. Pedigree J. P. N. (Vandepitte and Louis, 1955)

PEDIGREE J. P. N. (fig. 13)

$$z = \log \frac{2^8}{125} \{47\theta^3(1-\theta)^5 + 3\theta^4(1-\theta)^4 + 15\theta^4(1-\theta)^3 + 3\theta^4(1-\theta)^2 + 15\theta^5(1-\theta)^2 + 3\theta^6(1-\theta)^2\}$$

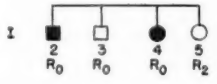

FIG. 14. Pedigree J. M. L. (Vandepitte and Louis, 1955)

PEDIGREE J. M. L. (fig. 14)

$$z = \log \frac{2^4}{163} \{78\theta(1-\theta)^3 + \theta(1-\theta)^2(2-\theta) + \theta^2(1-\theta)^2 + \theta^2(1-\theta)(1+\theta) + 78\theta^3(1-\theta)\}$$

These pedigree scores are given in table 1 and analysed in table 2. Had the linkage test been carried out sequentially with $\theta_1 = .10, .20$, or $.30$, taking the pedigrees in chronological order as they appear in table 1, pedigree 3 would have decisively

TABLE 1. PEDIGREE SCORES (z)

Pedigree	θ					$\hat{z}$	n[1]
	.01	.05	.10	.20	.30		
1	.2923	.2577	.2148	.1335	.0645	.30	2
2	−9.5484	−4.2320	−2.1820	−.5401	.0178	.17	22–25
3	5.0224	4.6821	4.2360	3.2641	2.1650	5.11	19
4	1.5604	2.3213	2.3170	1.9531	1.3763	2.36	13
5	2.4894	3.3039	2.7348	2.1087	1.2740	3.31	12–20
6	.2761	.2599	.2385	.1912	.1378	.28	3
7	.9919	.9084	.8053	.5972	.3799	1.01	4–9
R	2.6572	2.4420	2.1606	1.5538	.8915	2.71	11–12
B	−.1099	−.0908	−.0701	−.0380	−.0165	0	0–2
Ae	−7.3066	−3.8400	−2.3994	−1.0862	−.4551	0	11–15
S. S.	−1.4023	−.7212	−.4437	−.1938	−.0757	0	2
M. K.	−.3002	−.1007	−.0205	.0590	.0459	.06	0–5
J. P. N.	−4.0364	−2.0200	−1.2202	−.5311	−.2238	0	6–8
J. M. L.	−1.1179	−.4708	−.2351	−.0645	−.0135	.02	3–4
Total	−10.5320	2.6998	6.1360	7.4069	5.5681	7.42	
3 + 4 + 5 + R	11.7294	12.7493	11.4484	8.8797	5.7068	12.92	
2 + Ae + J. P. N.	−20.8914	−10.0920	−5.8016	−2.1574	−.6611	.05	

[1] n = the number of double backcross progeny in the pedigree.

TABLE 2. χ^2 ANALYSIS OF PEDIGREE SCORES

Source	d.f.	χ^2	P
Linkage	1	34.2	<.001
Heterogeneity among pedigrees	13	36.4	<.001
Among 3, 4, 5, R	3	2.6	
Among 2, Ae, J. P. N.	2	.6	
(3, 4, 5, R) vs. (2, Ae, J. P. N.)	1	25.6	<.001
Linkage in 2, Ae, J. P. N.	1	.2	
2(II.11, 12) vs. 2(excl. II.11, 12)	1	0.7	
2(excl. II.11, 12) vs. (3, 4, 5, R)	1	6.9	<.01

established linkage ($\sum z > 3$, $P < .001$). On the pooled data, linkage is enormously significant, with a significance level that for $\theta_1 = .20$ is strictly less than 1/antilog $7.4069 = 3.92 \times 10^{-8}$. For θ_1 distributed uniformly between 0 and $\frac{1}{2}$, the level of significance is less than $\left\{2\int_0^{\frac{1}{2}} \frac{f(y;\theta)}{f(y;\frac{1}{2})}\,d\theta\right\}^{-1} = 2.03 \times 10^{-7}$. By the large-sample test, $\chi_1^2 = 34.2$, $P < 10^{-8}$. There is no *association* between elliptocytosis and any one Rh blood type, and the segregation ratio for elliptocytosis, taking ascertainment into account, is not significantly different from 1:1 (Table 3). Therefore the evidence points to a true genetic linkage between the two loci.

The scores for $\theta_1 = .01$ and $\theta_1 = .05$ give an anomalous result. Omitting 7 small pedigrees with 5 or fewer double backcross progeny, the remaining 7 pedigrees fall unequivocally into two groups of close linkage and nonlinkage (fig. 15). Pedi-

TABLE 3. RH PHENOTYPES IN SIBSHIPS WITH AN AFFECTED PARENT. THERE IS NO EVIDENCE OF ASSOCIATION OF ELLIPTOCYTOSIS WITH ONE RH BLOOD TYPE

	R_1r	R_1R_1	R_1R_2	rr	R_2r	Other	Total
Affected sibs	35	19	16	11	6	14	101
Normal sibs	30	8	8	12	6	11	75
Total	65	27	24	23	12	25	176

$\chi^2_5 = 4.19, \quad .5 < P < .7.$

grees 2, Ae, and J. P. N. have scores less than -2, and therefore close linkage ($\theta < .05$) can be rejected at a significance level of less than .01. Individually and collectively, these pedigrees offer no evidence of linkage. On the other hand, pedigrees 3, 4, 5, and R have scores greater than 2, which suggest close linkage ($P < .01$ if $\theta = \frac{1}{2}$). This indication of heterogeneity is confirmed by the χ^2 test. Taking all 14 pedigrees, $\chi^2_{13} = 36.4$, $P < .001$; for the 7 largest pedigrees only, $\chi^2_6 = 28.8$, $P < .0001$. Heterogeneity within each of the two groups of large pedigrees is nonsignificant; between groups, it is highly significant ($P < 10^{-6}$).

Lawler and Sandler (1954) have suggested that heterogeneity between pedigree 2 and the pedigrees which show close linkage could be accounted for by illegitimacy in family II.11, 12, (fig. 2) which gives a $9r:1R_1^w$ segregation from the untested father, the 9th child receiving an R_1^w chromosome. Assuming legitimacy, the probability of such a sample or a more extreme one is 22/1024 = .021, which is perhaps no smaller than the probability that one of the last of ten children be illegitimate,

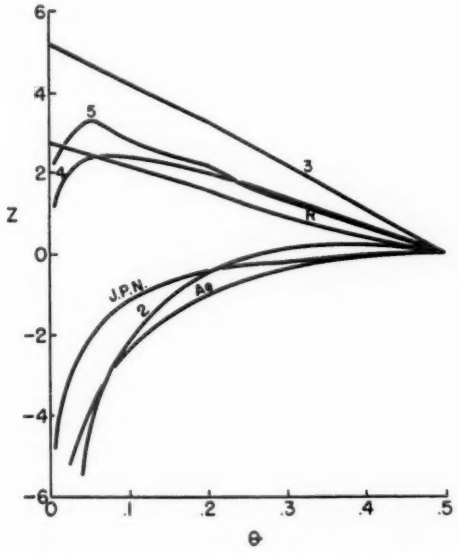

FIG. 15. Scores for pedigrees 2, 3, 4, 5, R, Ae, and J. P. N.

and the rest apparently legitimate. Since the ABO and other blood groups are consistent with single paternity, there is no real evidence for illegitimacy, especially as the recombination rate in the sibship II.11, 12 and the rest of the pedigree is homogeneous, and even with family II.11, 12 excluded, is significantly higher than in the pedigrees showing linkage but concordant with the other pedigrees showing no linkage. Finally, pedigrees Ae and J. P. N. are evidence for a class of pedigrees without linkage, and make a special hypothesis for pedigree 2 unnecessary.

As pedigrees 3, 4, 5, and R are apparently homogeneous, we can estimate the recombination fraction θ between the Rh and El loci. Because θ in these pedigrees is clearly very small, the estimate should be nearly unbiased, although a strictly unbiased estimate from an empirical separation of mixed data is possible only in the limit for large pedigrees. The distribution of Z as a function of θ is markedly skewed, but by trial and error it was found that the transform ξ = probit θ makes the distribution approximately parabolic. Using the points $\theta_1 = .01$, $\theta_2 = .05$, $\theta_3 = .10$, which are near $\hat{\theta}$, and substituting ξ_i for θ_i in equation 2, we obtain the following estimates

$$\hat{\xi} = 3.1684 \qquad \sigma^2_{\xi} = .0447 \qquad \hat{\theta} = .033$$

95% confidence interval: $.012 < \theta < .078$

Estimates from families of known parental genotype are in good agreement, although the variance is $\sigma^2_{\xi} = .1139$, and so these families give only 39% as much information as the full pedigree probabilities.

The number of certain recombinants is the least number that can account for the observed pedigrees. For sufficiently small θ, the frequency of certain recombinants is very nearly an unbiased estimate of θ, with an approximately binomial distribution (Haldane and Smith, 1947), and this can be made the basis of a separate estimate. There are 2 certain recombinants and about 60 informative progeny in these 4 pedigrees, hence the binomial estimate is

$$\hat{\theta} = \frac{2}{60} \pm \sqrt{\frac{(2)(58)}{60^3}} = .033 \pm .023,$$

in good agreement with the previous calculation. However, the corresponding 90% binomial confidence interval is $\theta < .086$, which is appreciably greater than the large-sample limit.

DISCUSSION

Since the distribution of the recombination fraction is clearly bimodal, it may be that there is a single elliptocytosis locus, which is closely linked to the Rh locus by an inversion in pedigrees 3, 4, 5, and R. This hypothesis cannot be excluded, but there is no critical evidence that inversion polymorphism is frequent in natural vertebrate populations (White, 1954), and it would be surprising if there were not selection against inversion heterozygotes in an organism with crossingover in the male. That man is of this type, is evident from the two certain recombinants in the pedigrees which show linkage; 4 III.12 (fig. 4) is from an affected mother, but 5

III.14 (fig. 5) from an affected father (by inference from the father's affected relatives). The occurrence of a translocation in half the pedigrees seems improbable and an explanation in terms of translocations or inversions requires ad hoc assumptions to account for apparent absence of linkage in some pedigrees and homogeneous close linkage in the remainder.

The hypothesis must therefore be considered, that elliptocytosis is not one genetic entity, but depends on two or more loci, say El_1 and El_2, the first of which (represented by pedigrees 3, 4, 5, and R) is closely linked to the Rh locus, while El_2 (in pedigrees 2, Ae, and J. P. N.) is in a different linkage group. A priori, this is not unlikely, as similar phenotypes often have different genetic bases. Medical and genetic studies have led to recognition of an increasing number of distinct abnormalities of the human erythrocyte (Neel, 1953), and presumably many more wait to be characterized. Thus Discombe (1948) has suggested on hematological grounds that spherocytosis is genetically heterogeneous, while elliptocytosis may vary among families in frequency and morphology of elliptocytes, hemolysis, anemia, survival of transfused erythrocytes, response to splenectomy, and other hematological findings (Motulsky et al., 1954; Wyandt et al., 1941). In conjunction with linkage data, more detailed hematological studies of these associated traits should be of interest, for intrafamily correlations exceeding $\frac{1}{2}$ would be independent evidence that elliptocytosis is not a single genetic entity (Haldane, 1941). Indeed, there has been so little comparative study of elliptocytosis pedigrees, that qualitative differences between the El_1 and El_2 phenotypes should be looked for. However, circumstantial evidence for or against the hypothesis of two elliptocytosis loci can be provided by observing whether large pedigrees continue to fall into a clear, internally homogeneous dichotomy in respect of linkage.

Recombination heterogeneity resulting from the confounding of two or more loci has several implications for the detection and analysis of human linkage. It is a serious obstacle to recognition of genetic carriers in small pedigrees (Neel, 1949), but a powerful tool in characterizing genetic, biochemical, and clinical entities. Heterogeneity gives special value to large bodies of data, either to detect linkage with power or to analyse it with precision, and thereby provides an additional argument for the use of polymorphic loci and rare dominants in linkage studies. Rare recessives, on the other hand, seldom provide the large kindred required for clear separation of heterogeneous material. Conclusions based on small numbers of families may be misleading when the data are not homogeneous, and the sample number (hence θ_1 in sequential tests) may have to be discouragingly large to detect linkage.

Analysis of genetic heterogeneity presents special difficulties in human data, because current homogeneity tests are exact only in the limit for large samples. The maximum likelihood (M.L.) test is liable to great inaccuracy in small samples, and has been shown to give spurious evidence for heterogeneity of linkage data for ABO and Friedreich's ataxia (Haldane, 1946) and to exaggerate evidence for heterogeneity of linkage between ABO and the nail-patella syndrome (Renwick and Lawler, 1955). The likelihood ratio (£) test gives a nonsignificant result in the first case, and a barely significant result in the second ($P \simeq .045$). Furthermore,

the nail-patella pedigree E, which shows no recombination and tremendously inflates χ^2 by the M.L. test, is less discordant by the £ test than pedigree C, which shows excessive recombination. There is evidence that the likelihood ratio test is surprisingly reliable in small samples (Fisher, 1950), although the method of graphical interpolation must be used with discretion and barely significant results should probably be discounted.

The question naturally arises, whether probability ratio tests are superior to fixed-sample-size tests when the recombination value is not constant. To investigate this point, the properties of the sequential test and the Fisher-Finney u score test have been determined for double backcross sib pairs, under the condition that both tests have a significance level of .001 and a Type II error in homogeneous material of .01 at $\theta_1 = .30$. Such a u score test requires a sample number of 1134 sib pairs, but the sequential test requires on the average only 355 sib pairs at $\theta = \frac{1}{2}$ and 529 sib pairs at $\theta = .30$ (Morton, 1955). For θ distributed uniformly between 0 and $\frac{1}{2}$ in different tests, the average sample number of the sequential test is 362 sib pairs, and the probability of detecting linkage is .71. The average power of the u score test is nearly identical (.70). Thus the sequential test has the same properties as a u score test based on 3 times as many data.

If we now alter the conditions so that for each sib pair nonlinkage and linkage with intensity θ are equiprobable, the sequential test has an average power of .59 for θ distributed uniformly between 0 and $\frac{1}{2}$ in different tests, and the u score test is virtually the same (.57). The average sample number for $\theta < \frac{1}{2}$ is 497, and since the prior probability of linkage is only about $\frac{1}{20}$, the expected sample number for the test is about 362, which is still only $\frac{1}{3}$ the number required by the u score test of the same strength. Thus the superiority of sequential tests over fixed-sample-size tests is apparently not altered by heterogeneity in the recombination rate.

SUMMARY

Probability ratio scores give simple sequential and fixed-sample-size tests for detecting linkage, a likelihood ratio test of homogeneity, and a maximum likelihood estimate of linkage.

Applying these methods, evidence for linkage between elliptocytosis and the Rh blood type is overwhelming on the basis of 14 pedigrees in the literature ($P < 10^{-7}$). By a sequential test against alternatives $\theta_1 = .10, .20,$ or $.30$, the fourth pedigree establishes linkage ($P < .001$). There is no association between one Rh type and elliptocytosis.

Linkage in these pedigrees is highly heterogeneous, both among all pedigrees ($P < .001$) and among the 7 largest pedigrees ($P < .0001$), which seem to fall into two internally homogeneous groups. Linkage is apparently absent in pedigrees 2, Ae, and J. P. N. However, pedigrees 3, 4, 5, and R show very close linkage, and the recombination value when linkage is present is estimated from these pedigrees to be $3.3 \pm 2.3\%$, with a 90% fiducial limit of 8.6%. It is suggested that elliptocytosis is not a single genetic entity in different pedigrees, but depends on either of two dominant factors, one of which is linked to the Rh locus.

Linkage studies have great value in the detection and analysis of genetic heter-

ogeneity, the recognition of which may help to resolve biochemical and clinical heterogeneity. The effect of recombination heterogeneity on linkage detection is discussed. Some evidence is given that likelihood ratio tests of homogeneity are more accurate in small samples than maximum likelihood tests, and that the greater efficiency of sequential probability ratio tests over fixed-sample-size tests is not altered by linkage heterogeneity.

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Psychiatric Aspects of Genetic Counseling*

FRANZ J. KALLMANN

Department of Medical Genetics, New York State Psychiatric Institute, Columbia University, New York 32, New York

PERHAPS IT IS partly because of the limited amount of counseling in human genetics in this country and partly because of consequent limitations in pertinent experience that workers in this specialized field are inclined to be on the conservative side. This guarded attitude seems to express itself in one of two ways. The first is a scientifically detached approach to all phases of counseling relevant to family planning. The other is an overcautious position based on the notion that eugenic considerations in this area are apt to create conflict and criticism and should therefore be kept apart from genetic considerations (Neel and Schull, 1954; Reed, 1955).

Hence the current emphasis in genetic counseling is placed on tendering a genetic prognosis, with no attempt made to appraise the advisability of parenthood. Decisions as to reproduction are regarded as falling strictly within the province of eugenics, and consequently are left up to the person or family seeking advice. The tacit assumption—often evidently wishful—is that intelligent people, who are morally entitled to truthful information regarding the prospects of their own health and that of their children, are capable of dealing with their family problems realistically and without special guidance.

From a psychological standpoint, realism may be defined as the tendency to view things as they are, rather than as what we want them to be. As a principle of adult behavior, this tendency is indicative of a mature mind and a wholesome pattern of adjustment to human imperfection and stress. However, a realistic attitude in the face of severe threats to health and survival can never be divorced from the existing circumstances. Nor can these circumstances be dealt with impersonally.

In no situation calling for genetic counseling can it ever be taken for granted that the person is realistic or will be able to attain a realistic attitude without the counselor's help. The attainment of this goal is highly desirable, but often requires considerable effort. Even well-educated persons may fail to achieve it without encouragement and guidance. In many instances, unguided reality enforcement is apt to create fear, anxiety, and inner tension, and not realism.

Frightened people either withdraw from reality by regressing to immature patterns of behavior, or they attempt to neutralize reality by means of pathological defense reactions such as repression and displacement, rationalization and projection. The final outcome will not be on the positive side, and an emotionally disturbed mind is a high price to pay for meek reality testing.

Psychological damage of equal severity may result from disregarding the emotional

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needs of the individual when parenthood seems contraindicated. For instance, merely because voluntary sterilization does not seem more objectionable on moral grounds than the practice of birth control is not sufficient reason to recommend it to persons who cannot know its future implications. Once again, we are dealing with a situation pitting intellect against emotions, where formal acquaintance with standard textbook descriptions of possible complications does not guarantee the ability to meet the actual experiences successfully. What is more, a sterilizing operation, even when performed without undue persuasion and conferring an important personal gain, such as eliminating the fear of pregnancy, is certain to have psychosomatic sequelae, if only because of the finality of the procedure (Ekblad, 1955).

Lacking stable life conditions or a reasonably defined motive—prerequisites which defy both analysis by abstract methods and expert advice proffered by mail—sterilized adults of either sex are in danger of responding to their lost reproductivity with feelings of inadequacy and insecurity. Too often such persons become fearful and apprehensive and it takes no formal psychiatric training to understand that a chronic anxiety state, brought about by the fear of developing the very gene-specific illness which is to be forestalled for the good of the family, may be as distressing and disabling as the disease itself.

The need for combining counseling with a personalized guidance effort is apparent throughout the field of medical genetics. It applies especially to inheritable traits which have a relatively late manifestation period and pose a real or imagined threat to the individual's health and adaptiveness, thereby affecting the emotional climate of the whole family. It also applies to defects such as early blindness or deafness, which are conducive to the formation of certain personal and intra-family patterns of adjustment highly specific to the impairment. The existential conditions of handicapped groups are rarely stabilized. It is unfortunate, therefore, that emotional deprivation in these groups is often aggravated needlessly. It is an erroneous belief, for instance, that these people live in a sheltered world and thus require less, rather than more, adequate provision for expert guidance (Kallmann, 1955).

Two recent observations, which can readily be multiplied from previous data, may serve to illustrate the significance of psychiatric problems encountered in genetic counseling. The first was a case that came to us through a surgeon in a rural district of New York State. He requested information about the advisability of an elective sterilization procedure in a thirty-three-year old woman who was desperately worried about the recent confirmation of a history of Huntington's chorea on her maternal side.

The young woman, a popular college graduate and happily married to a successful lawyer, was the mother of two healthy children, seven and nine years of age. Although she had not been pregnant for the past seven years, she was anxious to be sterilized. Deeply impressed by a letter from a counseling center, she felt obliged to follow the example set by her older sister who had voluntarily had herself sterilized.

When the young lady came to see us in response to our request, she presented the symptoms of an acute anxiety state, but revealed no evidence of a neurological disorder. Bravely resigned to the idea of being already afflicted with Huntington's chorea in its pre-clinical form, she produced the counseling letter which read as follows:

"It is generally accepted that Huntington's chorea is transmitted as a simple dominant characteristic. In this case, the probability of an individual being affected when one parent is affected is 1 chance in 2. This would be the situation which would apply in your case."

The letter went on to say, "We recognize certain additional factors associated with the disease, which may be of some value in your case. It is well established that the onset of the disease tends to be at approximately the same age among related individuals. This may be translated into the following risk of the disease appearing in your children: until such time as you develop Huntington's chorea or successfully pass the age of onset characteristic in your family, the probability that each of your children will be affected is 1 in 4."

The letter further stated, "It is important to note that in the event you should develop Huntington's chorea, then the probability that your children will be affected will be the same as your own present probability, namely 1 in 2. . . . Unfortunately, at the present time we know of no way of detecting an individual who will develop Huntington's chorea in time to prevent that anxiety associated with the disease and the anxiety with respect to one's children."

The unmitigated tenor of this communication speaks for itself and requires no psychological interpretation. At the time of our examination it was apparent that the young woman needed reassurance more than anything else. She herself confirmed this impression by the comment she had jotted down in the margin of the letter: "Hope is what we want—not statistics."

With all due respect to science, I do not hesitate to underscore the idea implicit in this young woman's remark. Adherence to scientific principles in genetic counseling is fine, but it is compatible with a certain amount of gentleness and psychological understanding in dealing with human problems.

As to appropriate medicogenetic indications for an elective sterilizing procedure, they should be compelling, fair and humanized. Without adequate motivation, the necessity for the operation will be rationalized in one way or another. Fearful conviction of inescapable ill health may associate itself with feelings of guilt and inferiority, or breed promiscuous tendencies by creating the need for acting out the problem (Knight and Friedman, 1954).

The second case was that of a deaf couple in their late thirties, one of many we have seen in recent months. Both had lost their hearing in early childhood, and both had been married before. One marriage had ended in the suicide of the mate, the other in divorce. The woman in this case had been married to a hearing cousin, a shiftless man with whom she had a hearing daughter. Her present husband had two deaf children by his former marriage to a deaf childhood friend of borderline intelligence. In his second marriage he had hoped for a hearing son, but instead his wife had borne him a deaf daughter. Curiously enough, the most unhappy and poorly adjusted of the household's four children was the hearing girl. She blamed much of her frustration on being continuously rejected by the deaf members of her family.

The genetic information given this couple prior to their marriage had been to the effect that their future children would probably have no hearing impairment in view of the fact that the half-sister's hearing was normal despite the cousin marriage of

her parents. Another clinic had offered the opinion that hereditary deafness tends to be recessive, generally occurs in sporadic form, and should preferably not be spread through marriage into a hearing family.

Here again, apart from the obvious need for scientific accuracy, it is evident that counseling involves people and not abstract problems. What is often overlooked in a genetic counseling situation of this kind is that even the best-educated people with an early hearing loss require instruction in the management of their family problems far more urgently than they need a genetic prognosis. Without special education, a deaf person is doomed to pass his life in a state of inarticulate ignorance and social isolation—without speech, without skills, and without the capacity for meeting the emotional and intellectual demands of adult life (Best, 1943).

If fully educated and trained, the deaf individual may be able to adjust to his particular conditions of minority group living, but he should not be expected to solve his specific family problems without expert guidance. In many instances, especially under conditions of gross parental immaturity or social inadequacy, it is certain to be as frustrating for hearing children as for non-hearing ones to be the offspring of deaf parents.

At the risk of accentuating the ambivalent attitude prevailing among some professional groups toward the aims and scientific principles of psychological medicine, it cannot be stressed too often that emotional disturbances may render people just as ineffectual as do any other serious illnesses. It is unfortunate that even in modern societies the psychic factors in many physical ailments continue to be neglected (Dunbar, 1954; Strecker, 1945).

Definitely avoidable, however, are situations where severe harm is done to relatively well-adjusted persons seeking advice in matters of health or family welfare. To this end, every professional worker—especially those whose work entails direct contact with human beings—should be made aware that thoughtless remarks or strenuously realistic comments may have a devastating effect on the adjustment and working capacity of sensitive persons, to such an extent that they may become chronic invalids.

It should be borne in mind that under the impact of such an experience even intelligent and ostensibly self-reliant persons may develop a feeling of living in an alien world. As a result, they become gloomy and insecure in their personal relationships, and gradually revert to immature forms of behavior.

In holding to one of the oldest ethical principles which dictates that mankind shall not suffer unduly and shall be given relief when it is within our power to do so (Lemkau, 1955), we must learn to accept the fact that proficiency of counseling in human genetics cannot be attained without some solid understanding of human psychology and human relations. To be sure, the primary responsibility of a genetic counselor is not specifically the mental health of the population with which he deals. Nevertheless, as has been indicated, he can do much good in this direction. He may be proud to consider himself a member of the "related service professions" working in the public health field, even as the family doctor, the school teacher, the probation officer, or the policeman on his beat (Ridenour, 1955).

While the scientific accomplishments of our discipline are truly remarkable, there

is little doubt that our training programs have yet to prepare future genetic counselors for activity in the public health field (Herndon, 1955). It stands to reason that these workers will be unable to take a real interest in human problems until they have been effectively oriented toward them. Perhaps, in order to have a conception of the scope of physical and mental illness, every student of human genetics should be afforded the opportunity to have meaningful experiences in dealing with troubled people (Kallmann, 1952). In this way he will learn how to talk *with* people, rather than at them or about them. Above all, he will gain a philosophy of public health. Then and only then will he know how to avoid creating ill will or ill health in any phase of his counseling work.

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A Further Study on the Familial Aspects of Carcinoma of the Stomach[1]

CHARLES M. WOOLF

Laboratory of Human Genetics, University of Utah

INTRODUCTION

WITHIN recent years a number of propositus studies have been carried out which were designed to investigate the importance of heredity as an etiological factor in human cancer. The usual procedure has been to obtain a measure of the frequency of cancer cases, or the number of deaths due to cancer among the relatives of a series of individuals (propositi) who were afflicted with a specific type of cancer and then to make comparisons with a control sample or with morbidity or mortality rates for the general population. A diversity of methods have been employed (reviewed in Woolf, 1955). Cancer of the breast has been investigated by Jacobsen (1946), Smithers (1948), Penrose, MacKenzie, and Karn (1948), Macklin (1953), and Woolf (1955); cancer of the stomach by Hagy (1954), Videbaek and Mosbech (1954), and Woolf (1955); cancer of the esophagus by Mosbech and Videbaek (1955); and cancer of the uterus by Brobech (1949), and Murphy (1952). In addition to these studies the literature also contains numerous references to others. Reviews and summaries of the most important of these can be found in Jacobsen (1946) and Woolf (1955).

There are differences of opinion among some of these authors as to the role genetics plays. The consensus of these studies, however, is that certain types of cancer such as carcinoma of the stomach, breast, and uterine body are influenced by genetic factors, but that certain other types such as carcinoma of the cervix and esophagus do not have a demonstrable genetic component. There is also controversy among some of the recent studies as to the quantitative importance of the genetic component when present, and whether for breast cancer it is organ specific or predisposes to cancer irrespective of site (see Clemmensen, 1949; Woolf, 1955). The discrepancies found among some of the results of these statistical studies are not all due to the errors inherent in random sampling but can be brought about because of the difficulty of obtaining accurate family data in regard to cancer and suitable control material. Many extraneous factors are known that can influence the results of a study of this type, but unfortunately, too little information is known about others. One source of bias, as well as the major reason why these studies are prolonged in time and require a great deal of effort, is the human element involved. Data are usually obtained by the interview method. A series of cancer patients or close relatives of the patients are interviewed and genealogical and medical information is requested about their family members. A similar approach is used for obtaining information about the families of

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a series of controls. The controls are usually free from cancer and selected from a specified segment of the population. Great variability exists among propositi and controls in their ability and willingness to give pertinent information concerning their families. Completeness of information is correlated with the educational status and general background of the informants (see Macklin, 1952), and it has been shown by Stocks and Karn (1933) that females tend to give more complete information in regard to other cases of cancer in their families, as well as genealogical data, than males. Propositi and controls often do not know if their relatives were afflicted with cancer and they seldom have reliable data on the principle sites of tumors in relatives.

The ideal study would be one in which the medical history of each of the relatives was carefully reviewed by conferring with physicians and examining hospital and pathological records. However, since this becomes a formidable task, the procedure in many studies is merely to check the medical histories of the relatives who were cancer suspects as suggested by the propositi and controls. There is a possible source of error in this method. A control might not give as complete a history for other cases of cancer in the family as does a cancer propositus or a relative of a cancer patient. Busk (1948) has suggested that a person afflicted with cancer is more conscious of other cases in the family than is a person who is free of the disease. Therefore, from the beginning a bias may be introduced by investigating only the individuals suggested by the informants. A significant difference could exist between the families of the cancer propositi and the controls even in the absence of an inherited tendency.

Another example of how the human element can enter into a study of this type is when records are not available to verify the belief of a propositus or a control that a relative was afflicted with cancer. Oberling (1944) has pointed out that the outcome of such situations can be greatly influenced by subjective factors. The investigator often has nothing to go on in these cases except the opinion of the informants and the symptoms described to him. His interpretation of these symptoms could be colored by his predetermined belief concerning the heritability of cancer.

It was conceived that it would be of interest and informative if family histories for a propositus study could be obtained without depending on the interview method. If an objective method could be realized in classifying the relatives and controls with respect to cancer, many of the subjective factors inherent with the human element would be eliminated. Facilities are available in Salt Lake City, Utah, for such a study. These consist of family group records on file in the archives of the Genealogical Society of the Church of Jesus Christ of Latter Day Saints (Mormon) and of death certificates for the state of Utah. The use of death certificates is not completely desirable. However, due to the unavailability of medical records in many cases, death certificates can be used to good advantage, especially for tumors with poor prognosis, when their limitations are realized and the investigations are planned accordingly.

In a recent publication by the present author (Woolf, 1955), the familial aspects of carcinoma of the stomach were investigated. The method of study was conventional in that family histories were obtained by interviewing some family members and by corresponding with others. Carcinoma of the stomach was also chosen to be studied by the present more objective approach. This was considered desirable since it would offer an opportunity whereby the results of two studies, on the same disease, that

were carried out by the same investigator by two different methods of approach could be compared. The present study is completely independent of the former as far as procurement of propositi and control material is concerned.

PROCEDURE AND RESULTS

Propositi for the present study were picked from the death certificates for individuals who died in the state of Utah between the years 1930 and 1949. Individuals were selected only when there was substantial evidence on the death certificate, as reported by the attending physician, that carcinoma of the stomach was actually present. The ideal situation would have been to choose propositi who were proven stomach cancer cases as evidenced by microscopic investigation of the suspected tissue. This was not feasible since the large hospitals in the state of Utah, which treat the majority of cancer cases, are privately owned and their medical files are closed to research of this type. A case can be reviewed only with the written consent of the patient or next of kin and the attending physician. The records of the Salt Lake General Hospital, operated in conjunction with the University of Utah Medical School, as well as other charity hospitals in the state, do not contain a large enough number of recent cases of stomach cancer to justify using the records for a study designed in this manner.

A further selection was made among the death certificates so obtained for white persons born and raised in Utah or in the immediate intermountain region. Included were some who were born elsewhere but who had spent most of their lives in the state.

Upon obtaining the names of a large series of individuals who died from stomach cancer, the next step was to take the names to the archives of the L.D.S. Genealogical Society in Salt Lake City, Utah. The Mormon church encourages its members to take an active interest in genealogical research. The Genealogical Society in Salt Lake City is the center of this activity. On file in this office are records that are of value for a study of this kind. These are referred to as family group records. Each record contains space for the entry of the names of a husband and wife and all their offspring, dates and places of birth for all these persons, names of the spouses of the offspring, dates and places of death for the parents, dates of death for the offspring, plus other information. These records are filed alphabetically based on the surname of the husband. Over three million of these records are on file. They are placed in the archives by members of the church and therefore deal mainly with Mormon families; however, parts of a large kindred may be on file even though only a few of its members are affiliated with the church.

A survey was made of the records to see if information was available for the families (parents and full sibs) of the individuals who died of stomach cancer. The names of the fathers of the individuals were used to enter the records. A family group record was available for approximately one-fourth to one-fifth of the names received from the death certificates. The names of the sibs and parents, pertinent dates, and places of death were copied from each record that was found. The records are very accurate for names and dates and places of birth, as well as names of spouses, but they are not always up to date on the dates and places of death for those deceased.

Upon obtaining the genealogical data the next procedure was to return to the Utah

State Division of Vital Statistics. With the aid of the cross index file for death certificates, a careful search was made for the names of the parents and sibs of the individuals who died from stomach cancer. Death certificates have been filed in this office since 1905. If a relative died in Utah since that time the certificate was usually found. However, the certificate was not found if the relative died previous to 1905, had a change in name, died in some other state, or was still alive. The study was only concerned with those relatives who have died in Utah since 1905. As soon as a certificate was located for a relative, notes were made as to the cause of death and date and age of death. The study was continued until histories were obtained of 300 families for which at least one death certificate in addition to that of the propositus was seen.

The control material used for a basis of comparison consisted of the frequency of deaths due to stomach cancer and other types of cancer among individuals of the same sex who died the same year in the same county at approximately the same age as each of the deceased relatives of the propositi. Controls were easily obtained since death certificates in Utah are filed by date of death and county, and bound into books. When a death certificate was found for a relative of a propositus, it became a matter of referring to succeeding certificates until the first qualified control was found. If the next succeeding certificate was a white person, of the same sex, and approximately the same age as the relative of the propositus, he was used as a control and the cause of death was noted. In order to be of approximately the same age a control had to be within the range set off by the relative's age plus or minus five years. If the next certificate did not qualify, as was usually the case, then it became a matter of turning the pages of the book until the first qualified control was found. Controls were obtained in a completely objective manner.

The death certificates for 173 fathers, 168 mothers, 390 brothers, and 260 sisters were observed, giving a total of 991. Of this total, 66 had cancer of the stomach listed as the cause of death, as compared with 32 in the controls. Chi square computed from a 2×2 table is equal to 11.7 (P is less than 0.001). Ninety-four deaths due to cancer other than stomach cancer were observed in the relatives and 82 in the controls. The

TABLE 1. COMPARISON OF THE NUMBER OF DEATHS DUE TO STOMACH CANCER AND OTHER TYPES OF CANCER IN THE FAMILIES OF THE PROPOSITI AND THE CONTROLS

Relation	Death Certificates Observed	Deaths Due to Stomach Cancer		Deaths Due to Other Types of Cancer	
		Families of the propositi	Controls	Families of the propositi	Controls
Father	173	5	3	12	8
Mother	168	11	4	7	14
Brother	390	38	17	37	28
Sister	260	12	8	38	32
Total	991	66	32	94	82
		$\chi^2 = 11.7$* $P < 0.001$		$\chi^2 = 0.74$* $.30 > P > 0.50$	

* Chi square based on totals (one degree of freedom).

TABLE 2. COMPARISON OF THE NUMBER OF DEATHS DUE TO CANCER IN THE FAMILIES OF THE PROPOSITI AND THE CONTROLS

Type of Cancer	Relatives of the Propositi	Controls
Stomach	66	32
Breast	8	7
Large bowel	17	15
Small intestine	4	1
Uterus or ovaries	12	8
Liver or pancreas, or gall bladder	13	14
Prostate	7	9
Lung	5	5
Other types	28	23
Total	160	114

$\chi^2 = 8.6^*$, $P < .01$.

* Chi square based on totals (one degree of freedom).

difference is not significant ($0.30 < P < 0.50$). These results are summarized in Table 1.

The different types of cancer deaths occurring in the relatives of the propositi and in the controls are shown in Table 2. There is a significant increase in the total number of cancer deaths in the relatives as compared with the controls, but as mentioned above, the increase is largely due to the higher frequency of stomach cancer deaths. Other common types of cancer occur at about the same frequency in the relatives as in the controls.

DISCUSSION

The results of this study suggest, as have previous studies (Videbaek and Mosbech, 1954; Woolf, 1955), that the heritable component for stomach cancer is organ specific. There was a significant increase in the number of stomach cancer deaths in the relatives of the propositi, but a significant increase was not observed for other types of cancer deaths.

The possibility that the familial accumulation of stomach cancer cases is not genetic in origin but due to the presence of exogenous factors in the home environment should not be overlooked. Clemmensen (1949) has clearly pointed out the dangers of limiting a propositus study to sibs and parents of the propositi; it destroys the only possibility of estimating the path of inheritance and at the same time of excluding the effects of "false inheritance", that is, the effects of environmental factors in the home or social level of the immediate family. Eating and dietary habits are factors that are often considered when discussing etiological factors for carcinoma of the stomach, although there are contrary views of their importance (Iason, 1953). There is no question that such factors are common to many members of the same family, and are even superimposed over two and three generations. Videbaek and Mosbech (1954) conclude there is no evidence that exogenous factors alone would result in the increased number of stomach cancer cases in the families of their propositi. Further evidence that an apparent exogenous factor is not operating in the home environment of the stomach

cancer patients was shown by Woolf (1955). The frequency of stomach cancer and other types of cancer deaths in the spouses of 540 stomach cancer patients was no higher than in the general population. If an important etiological factor were present in the home environment it might be expected that spouses would show an increased incidence of the disease, unless, of course, the unknown environmental factor is most effective when exposure to it begins during childhood. In this case, the spouses who would not share a similar environment until adult life would not be expected to show as high an incidence of the disease as sibs or parents. There is no evidence that such a factor exists, but it would be of interest to test for its presence by studying families with foster children. So far, such a study has not been carried out.

Even though the nature of the exogenous factors remains an enigma, it is still clear that something other than a genetic component is involved in the occurrence of stomach cancer. The increased number of stomach cancer deaths in the immediate families of the propositi is significant, but it is still not striking enough to suggest that heredity is more important as an etiological factor than the combination of the unknown exogenous factors. This is further indicated from the results of unselected twin studies (Gorer, 1953). Concordance among monozygotic twins for stomach cancer, even though it is higher than for dizygotic twins, still does not occur at a high frequency. Videbaek and Mosbech (1954) suggest that the predisposition towards stomach cancer is inherited and that unknown exogenous factors may accelerate its development.

The control material used in the present study should be considered. The mean age of all the individuals in the controls was only a few months younger than that for the relatives of the propositi. It is known that stomach cancer has a marked change in incidence for different age groups. However, the difference between the relatives and the controls in this respect is so small that it would not be expected to bias to any extent the results of the study.

The families of the propositi were mainly Mormon families, while the controls were taken from the general population of Utah and therefore consisted of both Mormons and non-Mormons. If stomach cancer were more prevalent among Mormons than non-Mormons, it is apparent that, the control material would not be adequate. In fact, if the differential between the two groups were large enough it would be possible that this alone would explain the difference observed between the relatives and the controls. However, this factor is not operating. Approximately 75% of the residents of the state at the present time are affiliated with the Mormon church and consequently the majority of the individuals included in the controls would be Mormons; but more conclusively, unpublished data (quoted with his permission) of Mr. John W. Wright, Director of the Division of Vital Statistics for the state of Utah, show that in the state Mormons do not have a higher frequency of stomach cancer than non-Mormons. Consequently the control material is not biased in this respect.

The control material used in this study also has other qualities in addition to the ease with which it is obtained. Since the controls are matched for age, sex, year of death, and county in which death occurred, any effect due to irregularities in reporting causes of death in some counties in some years would influence both the relatives and control series alike. Furthermore, matching for counties partially neutralizes any rural-urban effect that might be operating. However, this is not complete because

even though the majority of the counties in the state are completely rural in form, several are composed of rural communities in addition to the larger cities. Also, many rural residents are in the large hospitals or living with relatives in the cities at the time of death.

The results of the present study are similar to those obtained in the previous study in that the increased number of stomach cancer deaths in the relatives was about twice the number for the controls. This indicates that subjective factors were not greatly influencing the results of the previous study. Two different types of controls were used. In the previous study the observed number of stomach cancer deaths in the relatives was compared with an expected number based on proportionate mortality rates determined for the general population of Utah, patterned after a method used by Penrose *et al.* (1948). The differential observed in both of these studies is relatively similar to the one observed by Hagy (1954) in his gastric cancer study, but is less than that reported by Videbaek and Mosbech (1954). The latter authors found that the frequency of stomach cancer cases in the relatives of the propositi was over four times as high as in a control sample. It is likely that proportionate mortality rates and the type of control used in the present study are subject to less error than a control sample and therefore, on the average, constitute a more severe basis of comparison; however, it is also likely that the differential observed by the present author in both studies may be biased slightly downward. Propositi in these studies were obtained from death certificates. Any error in the cause of death of the propositi as listed on these death certificates by the physicians would result in a lowering of the value of the differential, since in each case when this occurred the sibs and parents would no longer qualify as the relatives of a stomach cancer propositus. Yet this is not the whole case, since the propositi used by Hagy were all microscopically proven gastric cancer patients. Regardless of the magnitude of the differential involved, the evidence from all these studies is consistent in indicating that heredity plays a role in the occurrence of stomach cancer.

SUMMARY

A propositus study is described which was designed to investigate the familial aspects of carcinoma of the stomach. Propositi were obtained from death certificates for the state of Utah and family histories from the records of the Latter Day Saints Genealogical Society in Salt Lake City, Utah. Causes of death for the relatives (sibs and parents) were determined from death certificates for the state of Utah. The control material was also obtained from death certificates.

It is concluded from this study, as other investigations have also shown, that heredity plays a role in the occurrence of stomach cancer.

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ety in Salt Lake City, Utah, for permission to use their records in carrying out this study.

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A Note on the Genetics of Van der Hoeve's Syndrome, with Special Reference to a Large Japanese Kindred*

TAKU KOMAI, HIKOJU KUNII, AND YASUNOSUKÉ OZAKI

National Institute of Genetics, Misima; Sagaé, Yamagata Pref.; and National Institute of Public Health, Tokyo

INTRODUCTION

VAN DER HOEVE'S SYNDROME, sometimes called "Eddowe's" or "Adair-Dighton's" syndrome, is one of the best known hereditary abnormalities in man. The syndrome is manifested most commonly as blue sclera (blue sclerotic), which is frequently accompanied by fragility of bones (fragilitas ossium, osteogenesis imperfecta, osteopsathyrosis idiopathica, Lobstein's disease), and deafness. These three symptoms are sometimes called the triad of this syndrome. Certain other structural abnormalities such as, short stature, large head often with bossed cranial bones, hyperflexibility of joints, dental anomalies and congenital heart defects, occasionally accompany the cardinal symptoms. Most of the authors on the genetics of this syndrome hold the opinion that all these symptoms are pleiotropic manifestations of a single autosomal mutant gene. Extensive bibliography concerning the genetics of this syndrome may be found in papers by Conard and Davenport (1915), Bell (1928), Aschner and Engelmann (1928), Waardenburg (1932) Fuss (1935) Kramer (1934), Gates (1946) and Seedorff (1949).

This abnormality is not uncommon among Japanese, and more than seventy pedigrees have been published. One of the present writers, Kunii, published in 1929 and 1930 a pedigree of this syndrome comprising 22 cases. This paper, together with Togano's papers (1929 and 1930) on three other pedigrees, which were published nearly simultaneously, are widely known among foreign investigators, and often quoted by them. About ten years later, Kunii happened to find a distinctly blue-sclerotic girl, while he was conducting ophthalmological examinations of school children in a district of Yamagata Prefecture. He traced the pedigree of this girl, and discovered a large kindred containing many patients afflicted with blue sclera, bone fractures, and deafness. Next year, Ozaki joined Kunii and assisted in the diagnosis of patients and the work of following the pedigree. The kindred at that time consisted of 243 members of whom 127 were examined and 72 diagnosed as affected. In 1948 the kindred was jointly reexamined by Kunii and Ozaki. By that time, the kindred had been enlarged to 377 members, of these 142 were examined and 86 were diagnosed as blue sclerotics. In 1953, examination was carried out for the third time, also by Kunii and Ozaki. This examination has added 20 members with 10 new patients to the kindred. Thus, this kindred (Fig. 1) comprises 96 blue sclerotics of various grade.

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and many of these patients have bone fractures (marked with X), deafness (D), or dislocation (L), or torsion (T) of joint. Besides, some members have normal sclera, but have suffered from either bone fracture, dislocation or torsion of joint, or deafness. When these are counted as abnormals, the kindred comprises 106 abnormals, and makes, probably, the largest pedigree ever published for this syndrome.

In addition to this kindred, Ozaki discovered eight smaller kindreds of the same syndrome near Tokyo comprising 13, 2, 7, 2, 4, 5, 6, and 2 patients respectively. Komai also encountered a kindred among the residents of Misima and Numazu, which comprised at least 10 patients extending in three generations. He also obtained an unpublished pedigree of a kindred inhabiting Siga Prefecture, by the kindness of the investigators in Kyoto Medical College. This kindred has 25 blue sclerotics distributed in four generations, and of these 15 have bone fractures and 9 are deaf.

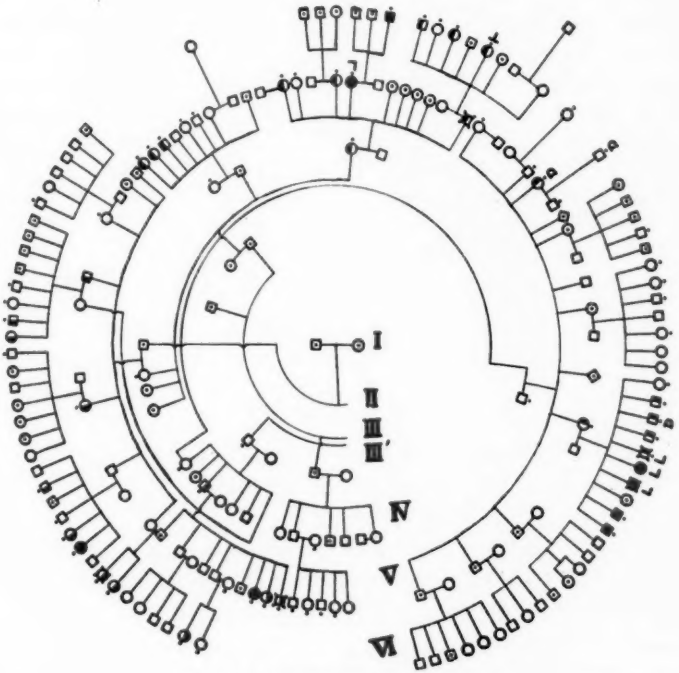

FIG. 1, PART 1. The Yamagata Kindred

Normal male and blue-sclerotic female of medium or high grade clinically examined

Male and female of unknown status

Slightly blue-sclerotic male and female, not examined

Blue-sclerotic male and female of medium or high grade with bone fracture

D Deafness

L Dislocation of joint

T Torsion of joint

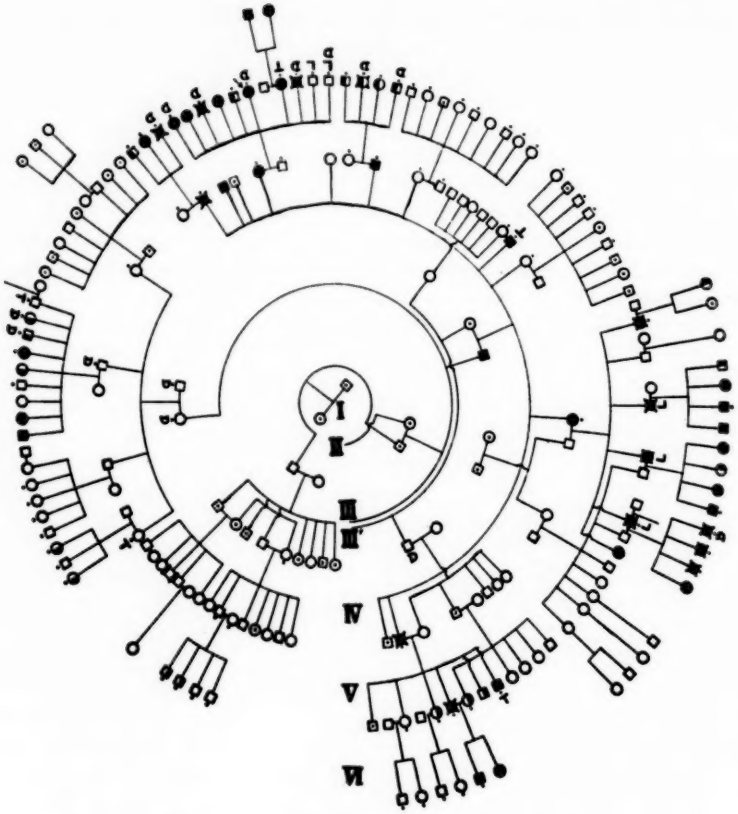

FIG. 1, PART 2. The Yamagata Kindred Completed
Symbols as in Fig. 1, part 1

The present paper deals primarily with the extensive Yamagata kindred mentioned above. However, the findings on other kindreds, as well as previous authors' discoveries, will also be referred to, when necessary.

THE YAMAGATA KINDRED

Among all the 106 patients, 52 are males and 54 are females. In the great majority of cases, the inheritance of the abnormality is direct, from a patient in the parent generation to the patients in the next generation, without distinction of the sex. Only in a few cases, apparently normal couples have had abnormal offspring. These findings indicate that the inheritance is due to an autosomal gene, and that its penetrance in heterozygotes is fairly high.

From 31 matings between abnormals and normals, 46 normals (22 males and 24

females) and 95 abnormals (46 males and 49 females) have been produced, besides 36 of unknown status. There is thus a significant excess of abnormals when a one-to-one ratio is expected. As mentioned already, many of the patients in this kindred were examined twice or three times, with a five or seven year interval between examinations. The patients were graded by the intensity of blueness of the sclera into "high", "medium", and "slight" classes. The intensity of each patient was judged independently by Kunii and Ozaki, and the judgements compared. Although this procedure could not be taken for all the patients, it served to some extent to avoid subjective judgement by the examiners. The intensity of blueness apparently showed no sexual distinction. It occasionally showed some difference according to age. We have encountered patients whose once distinctly blue sclera have become only slightly blue in the time between the successive examinations.

As a matter of fact, there is some variation in the color of sclera among normal individuals. Some people, especially young children, have sclera of a fairly intense blue color. So there is danger that such people among the kindred might have been mistaken for abnormals. Admitting the possibility of such mistakes, the normal to abnormal ratio among the progeny of normal $\times$ abnormal matings was re-examined, by putting the person with slightly blue sclera into the normal group, unless he had among his progeny an abnormal member. Persons who had had bone fractures, dislocation or torsion of joints, or who were deaf, were classified into the abnormal group without regard to the color of the sclera. According to this new scheme, 25 matings have yielded 55 normal (24 male and 31 female) and 65 abnormal (32 male and 33 female) progeny, a ratio falling well within the limit of 1:1 ratio ($0.5 > P > 0.3$).

Thirteen males and 7 females have had bone fractures, some, more than ten of them; 7 males and 8 females had had dislocated or distorted arm or leg joints; 12 males and 6 females, including some very young children, had difficulty in hearing. Several members had disproportionately large heads often with bossed cranial bones and short stature.

Besides the above 65 abnormals who were probably the progeny of matings of normal by affected, there are 3 (2 males and 1 female) blue sclerotics who are progeny of parents of doubtful genetic make-up as to the gene in question. The proportion of the individuals among the abnormals who had had bone fractures is 29.4 per cent; that of those who had had dislocation or torsion of joints is 22.1 per cent. Among the previous authors, Bell (1928) gives 63.0 per cent, Fuss (1935), 56.2 per cent, and Seedorff (1949), 84.0 per cent, as the proportion of individuals among the blue-sclerotic patients who had bone fractures. The frequency of deafness in our material is 26.5 per cent; this figure includes 3 old people. Bell gives 60.5 per cent, Fuss, 23.7 per cent, and Seedorff, 27.0 per cent, as the corresponding figure. The Siga Kindred, according to the Kyoto Medical College investigators, includes, among the total 25 blue sclerotics, 15 patients who have had bone fractures (60.0 per cent), and 9 (36.0 per cent) who are deaf.

Only 5 patients (4 males and 1 female 7.4 per cent) in our material have all three symptoms. The corresponding figure according to Bell, Fuss, and Seedorff is 43.8 per cent, 16.3 per cent and 26.0 per cent, respectively. The Siga Kindred has 28.0 per cent (7/25). Thus, on the whole, the Yamagata Kindred has a relatively low propor-

tion of other symptoms accompanying blue sclera. Still, there is no doubt that the abnormalities appearing in this kindred represent what are typical of van der Hoeve's syndrome, and the mode of inheritance is identical with that commonly accepted for this syndrome.

SOME REMARKS ON THE GENETICS OF VAN DER HOEVE'S SYNDROME

There seems to be little doubt that van der Hoeve's syndrome is due to a dominant autosomal gene. More properly, the gene belongs to the type of "conditionally dominant" (Levit, 1936) or "provisionally dominant" gene (Snyder and David, 1953), because the gene is known only in a heterozygous state, and no homozygote has ever been demonstrated. It is true that in some exceptional kindreds, only one, or two, of the three symptoms appear. Holcomb (1931) reports a family having bone fractures and deafness, but no blue sclera. In the Misima-Numazu Kindred no patient has difficulty in hearing. The occurrence of such kindreds does not affect the interpretation assuming pleiotropy of a single gene. It seems but natural that, because the manifestation of the gene in a heterozygous state will be much under the influence of other genes, the phenotype exhibits a wide range of variation.

The second question concerns the extent of the bone fragility of the affected members. In certain old terminology, the lesion appearing in this type of patient was called "osteopsathyrosis idiopathica" (Lobstein 1835). Besides this, a more severe type of bone fragility was recognized under the name of "osteogenesis imperfecta" (Vrolik 1849). This is manifested prenatally; the fetus has a soft membraneous cranium, and short and clumsy extremities with multiple bone fractures. It is still-born in many cases, or the baby lives for only a few days or weeks. This prenatal type had been separated, by some authors, from the commoner type of bone fragility and fractures, which appears in the post-natal period. It was Looser (1906) who first pointed out the nosological identity of the two types which differ only in the grade, and in the age of appearance of the deformity. He proposed to unify them under the name of osteogenesis imperfecta, and to distinguish them by calling them o.i. congenita and o.i. tarda, respectively. This view was supported by many subsequent authors, including Lovett and Nichols (1906), Sumita (1910), Zurhelle (1913), Bauer (1920), Knaggs (1924) Aschner and Engelmann (1928), Fairbank (1930), Kramer (1934), Seedorff (1949), and Grebe (1954). Authors like Bauer, Zurhelle, Knaggs, Rosenbaum (1926), Bierring (1933), and Seedorff have recorded cases where patients of these two types were born in the same family. Although it is doubtful whether all the cases reported as o.i.c. by certain of these authors, especially Bauer, may properly be assigned to this group, the cases recorded by Zurhelle, Knaggs, Rosenbaum, Bierring and Seedorff seem to warrant such classification.

These findings seem to support the view of the nosological identity of these two types, and strongly suggest their genetic identity. Seedorff distinguishes the o.i. tarda into two types, II and III, and calls them o.i.t. gravis and levis, respectively. The patients of type II are severely disabled at birth, and remain practically unimproved until death. Most of them have great difficulty in standing or sitting, and many are confined in bed throughout life. They rarely marry and have progeny. In the patients of type III, the fracture appears in a later period, and the fragility of bones often de-

clines with age. Nearly all of them get married, and are apparently as prolific as their normal relatives.

There is thus a complete series of the grade of bone fragility from the most severe form appearing in the intrauterine period to the phenotypically normal state. It is noteworthy, that the babies afflicted with the congenital type are predominantly female. Seedorff has found a single male among the seven cases encountered by him. He also cites Nielsen as stating the sex ratio to be one male to four females. This strongly suggests that the gene responsible for this type, often manifests its lethal activity in an earlier stage of intra-uterine life, especially in the male fetus.

As indicated above, all these different types of bone fragility seem to be due to the same gene, and its manifestation is under the influence of modifying genes. In some Japanese pedigrees of this syndrome, we find cases of type-II patients born of consanguineous parents who were blue sclerotic but without bone fracture, or who were both apparently normal (Tatibana 1926, Toyoda 1937—3 cases, Funaishi 1928, Kunii unpublished). The apparent sudden change in morbidity from the benign type to the severe type encountered in these cases is probably due to the decline of developmental homeostasis caused by inbreeding. Some cases of the production of a type-I (congenital) baby by a type-II or type-III parent recorded in the literature, are also perhaps due to a similar cause, namely, either the decline of homeostasis or the activity of modifying genes.

The writers are inclined to suggest another genetic interpretation for these different types, namely, that they are controlled by genes belonging to the same multiple-allelic series. The relatively clear distinction among those three types, in spite of the presence of some intermediate stages, seems to favor such a view.

Seedorff (1949) postulates three consecutive loci, *a*, *b*, *c*, all for bone fragility, adjacent to the locus of the gene for blue sclera, *bs*. He assumes that o.i.c. is due to the mutation of all these four loci, $bs + a + b + c$, while o.i.t. II is due to the mutation of $bs + a + b$, and o.i.t. III to the mutation of $bs + a$. Obviously, it would be very difficult to present sufficient evidence to test such a hypothesis.

The gene responsible for the congenital type obviously belongs to the lethal or sublethal group of human mutant genes. This was pointed out first by Mohr (1926, 1934, 1936), but has escaped recognition by many subsequent authors of human genetics. The gene is not included in Eaton's list of lethal genes in animals and man (1937). No information concerning the lethality of this gene is found in Gates (1946). Stern (1949) counts it among recessive lethals or sublethals. This view, however, is apparently incorrect, because the baby afflicted with this congenital abnormality is rarely the progeny of a consanguineous mating, and there seems to be no case, or at least it is seldom, that more than one such baby is born of the same parents. The gene is of a dominant lethal or sublethal type, like the gene for epiloia (Gunther and Penrose 1935), or for retinoblastoma (Falls and Neel 1951), or for multiple polyposis of the colon (Reed and Neel 1955).

ESTIMATION OF MUTATION RATE OF THE GENE FOR VAN DER HOEVE'S SYNDROME

The congenital type of bone fragility seems to appear each time as a new mutation. We can estimate, roughly, the rate of this mutation. According to Böök (1951) and

Kemp (1950, 1951), the incidence of births of this type of baby in both south Sweden and Denmark is about 2 among 100,000 births. In Japan also, this type of baby is extremely rare. A case has been reported by Katsu (1933). Mitani (1953) records no case of this abnormality among the 80,435 births in the Red-Cross Hospital in Tokyo. So we have to use the Scandinavian figures according to Böök and Kemp. Assuming that 4/5 of the male fetuses of this type die in an early stage and that no female fetus is lost, and if adjustment is made for this loss, then $(2 \times 1.67) \times 10^{-5} \times 1/2 = 1.67 \times 10^{-5}$ will be about the rate of gene loss per generation through this type of patient.

The patients of the type-II abnormality also rarely have progeny. The incidence of this type has never been estimated. Seedorff records 15 cases against the 7 cases of the congenital type. Although there is apparently a rather striking sexual disparity (4 males and 11 females) according to Seedorff's record of the patients of type II, the ratio lies within the limit of probability of the deviation from 1 to 1 ratio. So, if adjustment is made only for the prenatal death of male fetus in the congenital type, the number of cases of type II in Seedorff's record becomes 1.28 times that of the latter type. The rate of loss of the gene through patients of these two types may thus be estimated roughly at $(1.67 + 2.14) \times 10^{-5}$.

The great majority of the osteogenesis imperfecta patients belong to the mildest type, i.e. type III. The exact incidence of this type among the population has also never been given, so far as we know. Kunii in 1929 discovered a patient of this type among about 8,000 school children examined by him in Tokyo. Miyake, Niimi and Yoshida (1941) encountered 2 patients among the 75,150 residents in Nagoya; Oyama (1942) found one among 3172 villagers in Totigi Prefecture, and Kunii (1941, unpublished) encountered 3 patients belonging to the same sibship among about 20,000 school children examined by him in Yamagata Prefecture. These localities are rather widely scattered, so these figures could be used for the rough estimation of the incidence of the gene among the Japanese people. If the three patients belonging to the same sibship are counted as one, then the gene incidence based on these figures becomes $5/106{,}320 \times 1/2 = 2.35 \times 10^{-5}$.

The relative fertility (f) of the blue sclerotic patients was estimated from the data of the Yamagata, Siga and Misima-Numazu Kindreds, as well as from Kunii's, and Togano's earlier kindreds (1929, 1930). Only the progeny of mothers older than forty were utilized for the estimation. These included 31 sibships from normal $\times$ abnormal matings and 15 sibships from matings of the normal sibs of the abnormals with normal partners. The former sibships included 96 abnormal members, while the latter sibships consisted of 99 normal members. From these figures the value of f was obtained: $(96/31)/(99/(2 \times 15)) = 3.10/3.30 = 0.94$. Then the rate of loss of the gene per generation through the patients of this type may be calculated as: $2.35 \times 10^{-5} (1 - 0.94) = 0.141 \times 10^{-5}$. When this is added to the value given above for the other types, the total loss per generation becomes roughly 3.95×10^{-5}. If this loss is assumed to be thoroughly compensated for by new mutations, the same value may be taken as the mutation rate of the gene per generation.

There should be no need of pointing out that the above estimate is considerably handicapped by the inaccuracy of the basic data, still it will perhaps serve as the first approximation to the real value of the mutation rate of the gene responsible for this

abnormality. The greatest inaccuracy in the above estimate probably lies in the computed frequency of the type-II patient. The proportion of type-II to type-I patients given above is perhaps too low. If a higher value is allotted to this, the total loss, and consequently the mutation rate, will become higher. At any rate, it is unlikely that the rate will transcend the limit of the order of 10^{-5}.

SUMMARY

1. A large Japanese kindred of van der Hoeve's syndrome is recorded. The ratio of the blue-sclerotic patients afflicted with bone fragility and/or deafness is relatively low as compared with the ratio in the pedigrees recorded by previous authors on the same syndrome. Still, there is no doubt that all these symptoms are pleiotropic manifestations of a single autosomal dominant gene.

2. Assuming a common genetic background for all grades and types of bone fragility, osteogenesis imperfecta congenita, tarda gravis, and levis, the rate of mutation of the gene for this syndrome has been roughly estimated. As the basis of this estimate, the data for Scandinavian peoples were used concerning osteogenesis imperfecta congenita and tarda gravis, while the data for Japanese people were used concerning osteogenesis imperfecta tarda levis. The value of 3.95×10^{-5} has thus been obtained as the first approximation to the mutation rate of the gene for this syndrome.

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ADDENDUM

Dr. W. J. Schull has kindly notified the writers that his Atomic Bomb Casualty Commission associates did not find a single case of osteogenesis imperfecta congenita among the 76,626 newborn children in Hiroshima and Nagasaki examined by them, nor were there any among 8,900 births in Kure. If these figures are combined with Mitani's figure in the text (80,435), then there is no case of this abnormality among a total of 165,961 newborn infants in Japan. According to the figures for the Scandinavian peoples, there should be two or three such patients among these newborn. Whether the failure to observe any patients with o.i.c. is due to racial differences or not, can be decided only by further study. The great rarity of these babies is perhaps due, at least in part, to their death in a relatively early intra-uterine stage. At any rate, it seems to be very difficult, at the present stage of our knowledge, to get a correct figure for the frequency of this abnormality. We have made an adjustment for the sex disparity among such babies based on the observations of the Scandinavian authors. However, we do not know how closely the figure, thus obtained, has approached the real figure.

Also, the figures, presented by us, for the frequency of the common and benign type of van der Hoeve's syndrome in the Japanese local populations were originally intended only to give a very rough idea of the frequency of this type of patient among

the particular local population, without any regard to the difference between sporadic and familial cases.

Being based on such data, our estimates of the frequency of the different types of the syndrome, and consequently our estimate of the rate of mutation of the gene for the abnormality, cannot be accurate. Further study based on better data is highly desirable.

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A Family Study of Urinary β-aminoisobutyric Acid Excretion

STANLEY M. GARTLER

Institute for the Study of Human Variation, Columbia University, New York 27, New York

INTRODUCTION

β-AMINOISOBUTYRIC ACID (BAIB) is a non-protein amino acid excreted in human urine in widely varying concentrations from person to person, but at a relatively constant rate for any one individual. On the assumption that the variability between persons in the urinary excretion rate of this substance is described by a simple dimorphism (high and low excretors), two family studies of its variability have been carried out (Harris, 1953; Calchi-Novati, Ceppellini, Biancho, Silvestroni, and Harris, 1954). The results of these investigations are in agreement with the hypothesis that a single gene difference underlies the observed variability (high excretors being homozygous for a single recessive gene and low excretors either homozygous or heterozygous for the dominant allele). However, other data (Berry, 1953) indicate that the real nature of the distribution of the urinary excretion of BAIB may be continuous and not dimorphic as assumed in previous studies. This raises the possibility that the genetic picture is more complicated than indicated, and therefore it was felt worthwhile to undertake a more quantitative family study of this variable.

Unfortunately there is still much to be desired in the quantification of BAIB. Two-dimensional paper chromatography is the only technique available at present for measuring BAIB, and this method is subject to relatively large experimental errors, particularly when applied to urine. The only correction here, of course, is to increase the number of determinations for each sample. Further difficulties are presented by the fact that under different chromatographic conditions several other substances, known to occur in urine (histidine, methylhistidine, methionine sulfoxide, and some unidentified substances) may migrate to almost the same location as BAIB. In the case of histidine and methylhistidine color differences prevent misidentification, but their presence in relatively large concentrations does prevent accurate quantification of small amounts of BAIB. Other substances, such as methionine sulfoxide (probably present originally as methionine in urine but oxidized to the sulfoxide under certain conditions) can practically always be resolved by an overlap test (adding known BAIB to the urine sample and seeing whether the questionable substance and the known BAIB coincide).

MATERIALS AND METHODS

Families were collected on a volunteer basis, the qualifications being that both parents and at least one child be available for study, that the children be over 4 years of age, and that only healthy individuals be studied. In most cases three first morning

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urine specimens were collected. However, this was not always possible, and in some instances only one or two specimens could be obtained.

Creatinine concentration was determined for each urine sample (according to Bonsnes and Taussky, 1945) and a volume of urine equivalent to .040 mg. of creatinine was applied to Whatman No. 1 filter paper (23 cm × 27 cm) for two dimensional paper chromatography. The methods of Berry (1953), and Berry, Dobzhansky, Gartler, Levene, and Osborne (1955) were followed, using a phenol-buffer solution as the first solvent, a 2:6 lutidine-water mixture as the second solvent, 0.2% ninhydrin as a developer, and optical densitometry for quantification.

Where no BAIB was detectable on the first determination a second one was made, again using a volume of urine equivalent to .040 mg. of creatinine, and if BAIB was still undetectable, the specimen was considered to contain <.010 mg BAIB/mg creatinine. When BAIB was measurable on the first determination, from 2 to 5 more replications were run, usually varying the volume of urine applied, depending upon the level of BAIB in the sample. Standard sheets containing known amounts of BAIB were run with the unknowns at all times.

RESULTS

The distribution by age and sex of the urinary excretion of BAIB in the families studied is given in table 1. Though fluctuations are apparent, none show a consistent trend, and most important there are no significant differences between the two sexes or in the different age groups. In figure 1 the population studied is shown in histogram form. It appears that a dimorphism for BAIB excretion does not exist and furthermore, that there is little or no evidence of bimodality in the distribution. However, since the data, as will be shown, are still in agreement with the original mono-factorial hypothesis of Harris (1953), it must be considered, for the present, that the absence of a bimodal distribution is largely due to a combination of environmental and experimental variables, and to the relatively small sample size.

In order to test the hypothesis whether a single gene difference will explain our data, we must decide upon a dividing line between high and low excretors, Harris (1953)

TABLE 1. FREQUENCY DISTRIBUTION, IN PER CENT, OF URINARY EXCRETION RATE OF BAIB (MG BAIB/MG CREATININE) BY AGE AND SEX OF INDIVIDUALS

Age Group	Sex	No.	mg BAIB/mg Creatine		
			<.010	.010-.030	>.030
4–19	♂	43	67.4	25.6	7.0
	♀	36	75.0	19.4	5.6
20–39	♂	48	68.8	14.6	16.6
	♀	46	45.7	41.3	13.0
40–59	♂	38	68.4	26.3	5.3
	♀	41	68.3	19.5	12.2
60–	♂	18	44.5	44.4	11.1
	♀	12	58.3	25.0	16.7
Totals	♂	147	65.3	24.5	10.2
	♀	135	61.5	27.4	11.1

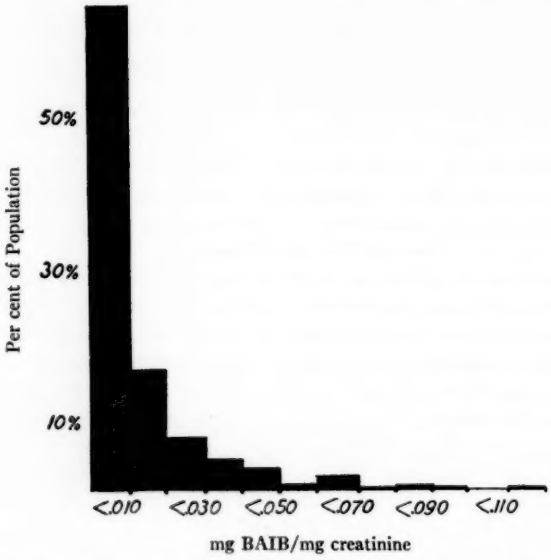

FIG. 1. Frequency distribution of the urinary excretion rate of BAIB in the population studied. Total sample = 282.

classified an individual as a high excretor, when the BAIB spot on the individual's chromatogram was darker than the alanine spot, or if the alanine spot was too faint, the glycine spot was used as a reference substance. He estimated that by this method, individuals classified as high excretors excreted from .070 to .200 mg BAIB/mg. creatinine.

The absence of a definite trough in our distribution makes classification into high and low excretors equally arbitrary. If .070 mg BAIB/mg creatinine is used as a dividing point, less than 2 per cent of the population (5 individuals) fall into the high excretor class; a considerably lower percentage than the 9.6 per cent observed by Harris (1953). Because the methods of quantification used by Harris (1953) differed from those employed in this study, it is not clear whether differences in the excretion

TABLE 2. TEST OF HYPOTHESIS THAT HIGH EXCRETION OF BAIB (>.030 MG BAIB/MG CREATININE) IS INHERITED AS A MENDELIAN RECESSIVE. TESTED ACCORDING TO FISHER (1939) ON THE BASIS OF A GENE FREQUENCY OF THE RECESSIVE ALLELE (t) OF .32; (t) ESTIMATED FROM THE PARENTAL POPULATION

Mating	Class of Family	No. of Families		χ^2	d.f.
		Observed	Expected		
T_ × T_	All children T_	52	51.4	.07	1
	At least one child tt	5	5.6		
T_ × tt	All children T_	12	11.5	.07	1
	At least one child tt	5	5.5		

TABLE 3. FAMILIAL DISTRIBUTION OF BAIB EXCRETION (MG BAIB/MG CREATININE)

Family	Parents		Offspring						
	♂	♀							
1	<.010	<.010	<.010						
2	.056	<.010	.065						
3	.022	.018	<.010	.024	<.010				
4	<.010	<.010	<.010						
4a	<.010	.028	<.010						
5	.018	.040	<.010						
6	<.010	<.010	<.010	<.010	<.010				
7	<.010	.025	.022						
8	<.010	<.010	<.010						
9	<.010	<.010	<.010	<.010					
10	<.010	.041	<.010	.049	.017	.041			
11	.038	<.010	<.010	<.010	<.010	<.010			
11a	<.010	<.010	<.010						
12	.018	.054	.042						
12a	<.010	.042	.015						
13	<.010	<.010	<.010	<.010	<.010	<.010	<.010	<.010	<.010
13a	.015	<.010	.028						
13b	.022	<.010	<.010	.019					
13c	<.010	<.010	<.010						
13d	<.010	<.010	<.010	<.010					
13e	<.010	<.010	<.010	<.010					
14	.015	<.010	<.010						
15	<.010	<.010	.010						
16	.020	<.010	<.010	<.010	<.010				
17	<.010	<.010	.012	<.010					
18	.012	.012	.021	.015					
19	.026	.012	<.010	<.010	<.010				
20	<.010	<.010	.015	<.010					
21	.036	<.010	<.010	.014					
21a	<.010	<.010	<.010	<.010	<.010				
22	<.010	<.010	.012	.010					
23	<.010	.015	.012	<.010					
24	.010	<.010	.024	.018					
25	.022	<.010	.038	<.010					
26	.024	<.010	.022	<.010					
27	<.010	<.010	.012	.018	<.010				
27a	<.010	<.010	<.010	<.010					
28	<.010	.046	<.010	<.010*					
29	<.010	<.010	<.010	<.010*					
30	<.010	<.010	<.010	<.010					
31	<.010	.062	.022	<.010					
32	<.010	<.010	<.010	.013					
33	<.010	.013	<.010	<.010					
34	.030	.034	.022	<.010					
35	.014	.015	<.010	.060	<.010	.062			
35a	<.010	.034	<.010						
35b	<.010	<.010	<.010	<.010					
35c	.062	<.010	<.010	<.010					
36	.025	.018	.078	<.010	.082	.090	<.010	.085	
36a	<.010	<.010	.028						
36b	<.010	.082	<.010	<.010					

TABLE 3. *Continued*

Family	Parents		Offspring						
	♂	♀							
36c	<.010	<.010	<.010	<.010					
36d	<.010	.085	.026						
36e	<.010	<.010	<.010	<.010	<.010	<.010			
37	<.010	.014	<.010						
38	<.010	.036	<.010	<.010					
39	<.010	<.010	<.010	.030	.030				
40	.012	.030	<.010	.030					
41	<.010	.018	<.010	.010	.012	.020			
42	<.010	.012	<.010	<.010					
43	<.010	<.010	<.010						
44	<.010	.015	<.010						
45	<.010	<.010	.012	<.010	<.010				
46	.015	<.010	<.010	.062	.022				
47	.012	.035	.052	.052					
48	.046	.012	.041	.015					
49	<.010	<.010	<.010						
51	<.010	<.010	<.010	.011					
52	<.010	<.010	<.010						
53	<.010	.010	<.010						
56	.011	.013	.118	<.010					
58	.015	<.010	.030						
59	<.010	<.010	<.010						
60	.010	.014	<.010	<.010	<.010	<.010			

* Identical twins, counted as one individual.

rate of BAIB reflect anything more than methodology variation. However, it may be pointed out that the work of Berry (1953) and Sutton and Clark (1955) in this country are in general agreement with the relatively low frequency of high excretors observed in this study. A more reasonable dividing point for high and low excretors appears to be in the neighborhood of .030 mg BAIB/mg creatinine. This seems so since the observed distribution indicates that the actual division or trough, if it exists, would most likely not be less than .030. That this is a minimal point is further supported by the results of mating #34 (table 3). In this mating the parents have values of .030 and .034, while their offspring have values of .022 and .010, which indicates that the homozygous recessive genotype would have a value not less than .030. In table 2 the hypothesis of mono-factorial determination, assuming that all individuals having values >.030 are high excretors, is tested, and, as can be seen, the data are in agreement with this hypothesis.

It should be mentioned that the division point is not critical in that it could have been made at a higher level (e.g. >.070) and the data would still be consistent with a mono-factorial hypothesis. This, of course, neither disproves nor proves the hypothesis, but merely is a reflection of the relatively small sample size in terms of segregating families.

DISCUSSION

As was mentioned earlier, the absence of a definite trough in the distribution can be ascribed in part, at least, to various environmental factors. For example, a limited amount of day to day variation in the excretion rate of BAIB in normal individuals is a general phenomenon. Furthermore, since BAIB is most likely a breakdown product of nucleic acid catabolism, it is probable that in certain pathological conditions, a marked change in the excretion rate of BAIB could take place. There is some evidence for this (Fink, Henderson, and Fink, 1951; Pare and Sandler, 1954) although it should be pointed out that no obviously pathological cases were included in this study.

A more complicated genetic system, such as modifiers or multiple alleles could also account for the absence of a trough in the observed distribution, and unfortunately, in this limited study, it is not possible to exclude such complicating genetic factors. Clearly the genetic mechanism involved in BAIB excretion must be relatively simple, as mere inspection of the family data in table 3 indicates, (i.e. children in high excretor families always fall into two relatively sharp classes). But, complicating genetic factors, such as those just mentioned, could be involved and still not be detectable or excludable due to the small number of segregating families observed.

In order to check such possibilities a more extensive family study of urinary BAIB excretion must be carried out. However, it should be noted that Caucasoid populations are far from ideal for this purpose due to their relatively low rate of BAIB excretion. Far more profitable for such studies would be populations in which the excretion rates of BAIB are comparatively high. According to a recent anthropological study by Sutton and Clark (1955), the Chinese may represent such a group, and another anthropological study now in progress at this laboratory indicates that the Caribs of British Honduras may also be such a people. It is to be hoped that genetic studies of BAIB excretion in such populations will be planned, to follow up these valuable leads.

SUMMARY

In conclusion, this work has shown that while the distribution of the urinary excretion rates of BAIB is most likely continuous rather than dimorphic, as assumed in Harris' (1953) work, the data are still in agreement with Harris' hypothesis that the observed variability is largely determined by a single gene difference.

ACKNOWLEDGMENTS

The author is deeply indebted to Professor Th. Dobzhansky for his assistance and encouragement during the course of this work. He is also grateful to Mrs. T. Gidaspow for her excellent technical assistance and to Profs. L. C. Dunn and H. Levene for reading the manuscript.

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BOOK REVIEWS

Population Genetics

By C. C. Li, Chicago: University of Chicago Press, 1955, pp. xi and 366. Price $10.00.

In 1908, G. H. Hardy, an English mathematician, and W. Weinberg, a German physician, laid the cornerstone of population genetics, that branch of genetics which deals with the nature of hereditary differences in groups of individuals. They demonstrated that, in a randomly mating population, gene and phenotype frequencies were invariant provided the population is infinitely large, there is no selection for or against any of the genotypes involved, there is no mutation, and the generations are nonoverlapping. Forty years later (in 1948) the subject matter of population genetics was of sufficient scope to warrant the publication of three books, namely, G. Dahlberg's *Mathematical Methods for Population Genetics*, C. C. Li's *An Introduction to Population Genetics*, and G. Malécot's *Les Mathématiques de l'Hérédité*. While each of these books has contributed to the broader dissemination of the fruits of these forty years of inquiry, Dr. Li's book has enjoyed the greatest popularity in the United States. This popularity stems primarily from the fact that it is more admirably suited as a textbook, since it possesses a comprehensiveness not possessed by Dahlberg's effort, and does not require the level of mathematical maturity required by Malécot's book.

Thus far the theory of population genetics is due largely to three individuals, R. A. Fisher, J. B. S. Haldane, and Sewall Wright. Their interests have ranged over a variety of problems, among the latter being the rate of change of gene frequencies with natural selection, a theory of natural selection, the persistence of a mutant gene in a population, the consequences of various mating systems, and the steady-state distribution of gene frequencies under increasingly general conditions. To each of these problems and numerous others, Dr. Li addresses himself with familiarity. The results are almost invariably improvements, from the pedagogical standpoint, on the originals. A measure of this success is undoubtedly due to Dr. Li's device of interjecting, at various points, excursions into "pure" mathematics. That this device which could detract from the central genetic argument never does so, is a tribute to Dr. Li. The general impression which one carries away from a reading of this book is of a difficult job admirably done. It is a thoroughly excellent book, and one which can be highly recommended as a cogent and clear introduction to problems in population genetics.

After this endorsement, perhaps I may voice several differences in opinion with regard to emphasis, approach, or selection of topics. Firstly, *Population Genetics* places emphasis on the deterministic rather than the stochastic aspects of the theory of evolution. Whether this is an advantage or a disadvantage is a moot point indeed. It is to be hoped, however, that in the next edition Dr. Li will see fit to present some of the more recent work, such as Feller's on diffusion processes in genetics, and Kimura's on genic selection in a finite population. Secondly, somewhat more emphasis might be placed on important unsolved problems, and on what might be termed the reality of the models which we employ. The correspondence between a model based on genic selection, say, and the phenotypic selection which must be the more common occurrence in nature is certainly not all that one might desire. There exists, in the eyes of the reviewer, a real danger that the student, confronted with the mathematics required to exploit the simpler models, will lose sight of the reservations which should be attached to them. Thirdly, in the discussion of applications of the equilibrium law and "Snyder's Ratio," a reference to Fisher's approach to this problem would not be inappropriate, and in the discussion of sterility genes Finney's notions on isoplethy and unstable gene systems could have been mentioned.

The present version of *Population Genetics* differs in a number of particulars from the first. Among these are (1) the replacement of the chapter on "Segregation in Small Families" by a chapter on "Genetic Variance and Correlation," (2) the inclusion of numerous new problems, (3) a more detailed bibliography, (4) a variety of new, helpful figures particularly in the chapter on "Stationary Distributions of Gene Frequencies," and (5) the inclusion of some of the developments in the interval 1948–1954. These changes coupled with a much better printing job make the present edition a de-

cided improvement over the first. It is unfortunate, therefore, that the price of this book will lead many to content themselves with the earlier version.

To attempt to single out for commendation specific items in Dr. Li's book seems patently unfair because of its overall excellence, and the fact that the items selected will quite probably reflect only the reviewer's special interests. However, the comparatively small number of typographical errors was a pleasant surprise, and none of the ones noticed seemed to occur in a place where they might cause confusion although not all of the formulae were checked. The explanation of the theory of path coefficients continues to be singularly fine. The new chapter on genetic variance and correlation contains an ingenious device, namely, the use of matrices of conditional probabilities to obtain the joint frequency distributions for any type of relatives. Clearly this list could be longer; suffice it to say that this is an excellent book well deserving of a conspicuous place in the library of everyone engaged in research and/or in teaching genetics.

WILLIAM J. SCHULL
Heredity Clinic, University of Michigan

Theoretical Genetics

By RICHARD GOLDSCHMIDT. Berkeley, California: The University of California Press, 1955, pp. 563, $8.50.

THIS book constitutes a remarkable attempt to organize a large part of modern genetic thinking with the hope that it may "contribute to the emergence of a theoretical genetics". It is, in this day of review papers and compendia, a prodigious individual effort and thereby carries the great advantage of a single point of view. Although, it does have the disadvantage that one mind can hardly encompass all aspects of Genetics adequately, Dr. Goldschmidt comes to this task well prepared. By his own word, he has been doing genetical work for forty five years and the first of his papers referred to in the volume was published in 1903. Furthermore, his conclusions have often been unorthodox—his views concerning the nature of the gene, genic action and the origin of species have continually stirred up controversy. Thus Dr. Goldschmidt combines an unparalled background in Genetics with an imaginative flair tempered by much vigorous cerebration.

The subject of this book is an appraisal of the extent to which Genetics has answered three basic questions: "(1) What is the nature of the genetic material? (2) How does the genetic material act in controlling specific development? and (3) How do the nature and action of the genetic material account for evolution?"

Part I takes up the first question. It includes a very up-to-date review of biochemical findings about the molecular structure of genetic material. Here, Dr. Goldschmidt faces, with characteristic abruptness, the unresolved problem of whether DNA or protein is the genetic material. The discussion proceeds with a consideration of the genetic meaning of heterochromatin and concludes, as could be expected, with a monumental examination of the phenomenon of position effect. This last section contains a particularly imaginative rephrasing of McClintock's recent work on mutable loci in maize.

The second part is entitled, "The cytoplasm as seat of genetic properties". Here Dr. Goldschmidt interprets a sizeable array of evidence to indicate that the concept of the independent plasmagene is "tumbling" and further emphasizes that "it is not advisable to call mitochondrial (and plastid) conditions and their change cytoplasmic heredity". Although it may seem from these quotations that Dr. Goldschmidt's emphasis is largely semantic, it must be made clear that his interpretations primarily are toward the idea that there is no genetic material in the cytoplasm independent of the nucleus.

Part III, "The action of the genetic material," benefits, perhaps more than any other part of the book, from the span and breadth of Dr. Goldschmidt's view point. A truly amazing variety of evidence is brought to bear upon this problem. The discussion begins with a short account of Hammerling's classic *Acetabularia* experiments as a generalized example of the ultimate nuclear control in development. He then shows why he believes the "phenomenon of phenocopy the basic phenomenon for any study of genic action". Here he concludes that the explanation of phenocopies

and therefore gene action will be found in terms of "quantitative, kinetic actions rather than in terms of qualitative biochemical features". This puts into perspective the achievements of the biochemical geneticists and brings up the question of what, after all, is the "primary action" of the gene. He makes very clear how little is really known about how genetic information is transferred from the chromosome to the cytoplasm despite the recent hopes for RNA in this role.

The major chapter in Part III is devoted to the "Genic control of development". The discussion builds upon the basic idea that "the cytoplasm must become differentiated into regions which in some way are competent for specific genic actions, based upon primary or secondary genic products removed from the nuclei". There follows a detailed inquiry into what the facts of gene dosage and penetrance indicate about the nature of genic action. The last section is a high level treatment of the "four dimensions" of genic action in development.

Part IV dealing with the "Genetics of Sex Determination" is a succinct and unified development of the balance theory of sex determination.

Part V "Genetic Theory and Evolution" is very brief. It recapitulates Dr. Goldschmidt's thesis that neo-Darwinian ideas account only for the origin of species, not the higher taxonomic categories. Two aspects of his ideas on this subject are presented. First, that chromosomes themselves have undergone relatively little evolution, i.e. that very little new genetic material has been added to chromosomes. Second, that new taxonomic groups have arisen primarily as a result of a "repatterning" of genetic material already present. In this connection, several models of "macromutation" are given.

After reading this book few people will fail to be impressed at the expanse of material reviewed and interpreted herein. It should certainly become standard reading for all advanced students of Genetics. For Dr. Goldschmidt's function, as it has been often before, is to plow up the topsoil of genetic knowledge. Further, he plants a vigorous crop of ideas, some of which are certain to be harvested despite the rigors of the scientific climate.

BEAL B. HYDE
Univ. of Oklahoma

Evolution, Genetics, and Man

By THEODOSIUS DOBZHANSKY. John Wiley and Sons, Inc., New York, 1955, pp. ix + 398, $5.50.

THIS book was evidently written as a college textbook, designed to be comprehensible to the student with no more than the high school level of preparation in biology, and suitable for the sort of course often called "Genetics and Evolution", or "Evolution and Genetics". It should fulfill this purpose admirably, for it is clearly and interestingly written, but it should also do much more than this. The author points out in the preface that some "advanced" material is included, and a number of unsettled and controversial problems are discussed. As a result there are few workers in the sciences related to man, except perhaps professional geneticists, who will not have something to learn from a perusal of this volume.

The book is so well integrated that it is not easy to describe its various parts. Broadly, it deals with genetics, evolution, and man; exactly as the title implies. After a preliminary chapter on the nature and origin of life, three chapters are devoted to an extremely clear exposition of the elements of genetics. Later chapters deal with mechanisms of evolution, the concepts of race and species, and various examples of evolution. Human evolution in particular is discussed in considerable detail, and the book ends with a chapter on Chance, Guidance, and Freedom in Evolution which discusses ideas such as autogenesis, finalism, orthogenesis, orthoselection, and the bearing of evolutionary doctrines on ethics.

The bases of classification of species into races, especially of the human species, are very well discussed, and an account of human races, based mainly upon the ideas of Coon, Garn and Birdsell, is included.

The author makes a few errors of the type common to nonanthropologists. On page 153 we are told about the dolichocephalic heads among the northern and southern Europeans; examination of the maps in Coon's *The Races of Europe* will show that in fact these populations are mesocephalic.

It might also seem that too much is made of the great difference in mental ability which is supposed to separate man from the animals. Perhaps it is only sympathy with the underdog, but the reviewer feels that animals are a good deal smarter than we give them credit for, and that man's main advantage is the gift of articulate speech. But these are trivial points.

The publishers have, probably unintentionally, played a little practical joke upon the author. Prof. Dobzhansky is well known for his opposition to the views of the racists, and in the present work points out again that, in relation to people, judgments of superior and inferior have little meaning. But the dust jacket of the book has as illustration an evolutionary spiral; microscopic alga, coelacanth, Irish elk, australopithecine, and a genial but anemic looking individual obviously representing modern man. On page 353 we discover that this pinnacle of evolution is a "Northwest European". We may be sure Prof. Dobzhansky would never have approved this drawing.

This is without doubt the finest textbook of genetics and evolution now available, and its emphasis upon applications to man make it especially valuable. It is well printed, on good paper, and contains numerous excellent illustrations. There is an index.

WILLIAM C. BOYD
Boston University

Embryologie: Ein Lehrbuch auf Allgemein Biologischer Grundlage

By PROF. DIETRICH STARCK (Frankfurt am Main) Stuttgart: Georg Thieme Verlag, 1955, pp. 688; 522 figures. DM78.-

THIS is primarily a text on mammalian development but it is unique in its inclusion and integration of facts from comparative, descriptive and experimental embryology. Although it lacks the details of special treatments of these aspects of embryology, it is by no means superficial in any respect. The author prefers to think of it as a biologically oriented story of development and, indeed, one cannot avoid getting this impression from the wealth of information, gleaned from our knowledge of development in all areas of the animal kingdom, that he brings to bear on problems of development.

The book is subdivided into two parts for convenience in the presentation of General and Special Embryology. Its chief justification lies in the section on General Embryology which constitutes over half of the book. This emphasis is in strong contrast to comparable texts in English where general embryological considerations occupy about a third of the total space. The plan of presentation is typical; the story begins with germ cells and their formation and proceeds through fertilization, cleavage, etc. It is here, in particular, that the author makes his work distinguished by an interweaving of knowledge from the aforementioned artificial subdivisions of the science of embryology. Geneticists will appreciate that the fundamentals of their science are integrated in logical places. In connection with the presentation of meiosis, crossing over and chromosome mapping is explained. Following the part on fertilization is a section on the chromosome theory of inheritance which begins with the fine structure of the chromosome, and proceeds with cytological evidence of the presence and disposition of genes on the chromosome. Sources of variation in the hereditary material are discussed and the mechanism of gene action is considered. A separate section is devoted to sex determination. For an embryology text, this is an exhaustive treatment of the subject. Particularly outstanding are the sections devoted to placentation where the comparative aspects are emphasized though not to the exclusion of physiological considerations.

The presentation of the organ systems follows a pattern very similar to that found in most texts on mammalian development with the exception that there is somewhat more emphasis on comparisons with other vertebrates. Throughout the book the author takes opportunity to present theoretical considerations. This policy is exemplified in the final section of the book which he devotes to the consideration of the origin of the vertebrate body plan. An appendix is included in which the author gives a classification of the vertebrates and a table showing, mainly, the duration of the period of development in the vertebrate groups although for some groups additional data is included. There are three indices; a very useful listing of animals referred to in the text, an author and a subject index.

Most fault finding comes with the listing of the literature citations. All references are in the back

of the book listed alphabetically by author under general subject headings. These headings do not agree with the organization of subjects in the text which makes references difficult to find. This difficulty is further enhanced by the bibliographic technique in which one reference follows another with no break in continuity. No technique is employed to set one author's work conspicuously apart from others. This may effect an economy for the publisher but it wastes a lot of the reader's time and is conducive to error. One is also occasionally disappointed to find that although an authority is mentioned in the text no citation is provided in the bibliography.

The embryologists will welcome this book as an important reference work and a refreshing presentation of the subject.

RICHARD A. GOFF
University of Oklahoma

Mechanisms of Microbial Pathogenicity

Edited by J. H. HOWIE and A. J. O'HEA (University of Glasgow) New York: Cambridge University Press, 1955, pp. x + 333, $5.00.

THE genetics of resistance and susceptibility to pathogenic microorganisms constitutes one aspect of human genetics which is not well understood. One approach to this problem is through the study of pathogenic mechanisms, *per se*. This volume contains the papers presented at the Fifth Symposium of the Society for General Microbiology which was held at the Royal Institution, London in April, 1955. The papers are centered around the subject of the processes and mechanisms whereby infecting organisms produce effects in the host such as disturbances of function or metabolism, toxemia, visible lesions, or death of the host.

In reading the excellent papers in this book, each written by an expert in the field, one is impressed by both the mass of factual information already available and the vast amount of knowledge yet to be gained. The subject matter covers all types of microorganisms and both animal and plant hosts.

The compilation of this material in a well written, organized style based on fundamental concepts makes this volume yet another valuable contribution from the Society of General Microbiology.

J. B. CLARK
University of Oklahoma